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Innovative Methods for Estimating Densities and Detection Probabilities of Secretive Reptiles Including Invasive Constrictors and Rare Upland Snakes

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14. ABSTRACT This project demonstrates the broad applicability of a novel simulation-based technique for estimating density of secretive snakes through case studies of the imperiled southern hognose snake (<i>Heterodon simus</i>) in the North Carolina Sandhills and the invasive Burmese python (<i>Python molurus bivittatus</i>) in Everglades National Park, Florida. For both species, existing radiotelemetry and extensive road survey data are used to generate the first density estimates available for the species. The results show that southern hognose exist at relatively low densities (0.17 per ha), raising concern that this species may not only have declined in geographic range but may also occur at relatively low densities and/or be declining in their strongholds. Estimates of python density (1.5-5 per km ²) provide baseline information that is critical for management of this harmful invader. Assumptions and model parameters are discussed in the report and guidelines are provided for using this novel technique to estimate densities of secretive reptiles on DoD lands.					
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Background:

Accurate estimates of population density are a critical component of effective wildlife conservation and management. However, many snake species are so secretive that their density cannot be determined using traditional methods such as capture-mark-recapture. Thus, the status of most snake populations remains completely unknown, presenting a substantial obstacle to wildlife inventory or management plans and impeding effective management of these species on DoD lands. Here we develop a novel technique for estimating density of secretive snakes that combines behavioral observations of snake road crossing speed, systematic road survey data, and simulations of spatial movement patterns derived from radiotelemetry, without relying on mark-recapture. We demonstrate this approach through case studies of the imperiled southern hognose snake (*Heterodon simus*) in the North Carolina Sandhills and the invasive Burmese python (*Python molurus bivittatus*) in Everglades National Park, Florida.

Objective:

- 1) Develop a method to simulate snake movements to calculate detection probabilities during systematic road surveys
- 2) Use this method to estimate densities of southern hognose snakes and Burmese pythons.

Summary of Approach:

Our density estimation approach combines behavioral observations of snake road crossing speed, effort-corrected road survey data, and simulation-based modeling of spatial movement to estimate population densities. We used previously collected radiotelemetric data to quantify movement metrics (frequency, distance, and direction of movement in relation to home range center and roads). These data were then used to parameterize individual-based movement models in a biased correlated random walk framework to estimate the frequency with which individual snakes cross roads. Next, information on survey vehicle speed and snake crossing speed were used to determine the probability of detecting a snake, given that it crossed the road transect during a survey. We then used the resulting relationships between observation frequency and density to infer density from large databases of systematic road survey data for our focal species. Finally, we simulated models to assess sensitivity of our density estimates to uncertainty in parameter values and model assumptions.

Benefit:

Our research enhances strategic planning and management of DoD's natural resources by developing a novel framework for obtaining heretofore unavailable information (density) critical to management of species of conservation concern. Specifically, we develop a standardized, verified tool, applicable to many species, regions, and installations to ultimately assist with resource management and reduce or avoid training restrictions. Further, our case studies benefit the individual missions of military installations by providing baseline density information for our focal species, the southern hognose snake and Burmese python. This information will aid resource managers at bases tasked with conserving southern hognose snakes, or preventing establishment, spread, and impacts of invasive pythons.

Accomplishments:

We have developed a novel technique for estimating density of secretive snakes using road survey and spatial movement data, without needing to recapture individuals. We describe this method, through the first case study of the southern hognose snake, in a manuscript that has been submitted for publication in a scientific journal, and are following up with a manuscript reporting python density that is near submission. In addition to describing the method, these manuscripts provide the first density estimates for either of our focal species. We find that southern hognose exist at relatively low densities (0.17 per ha), raising concern that this species may not only have declined in geographic range but may also occur at relatively low densities and/or be declining in their strongholds, such as the North Carolina Sandhills. Our estimates of python density (1.5 – 5 per km²) provide baseline information that is critical for management of this harmful invader. Our results suggest that current control measures are insufficient to curb population growth and spread of pythons in South Florida, but highlight strong variation in python density over time. Overall, our method enhances our ability to study and manage many secretive snake species that are most effectively sampled using road surveys.

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Executive Summary

Accurate estimates of population density are a critical component of effective wildlife conservation and management. However, many snake species are so secretive that their density cannot be determined using traditional methods such as capture-mark-recapture. Thus, the status of most terrestrial snake populations remains completely unknown, presenting a substantial obstacle to wildlife inventory or management plans. Here we develop a novel simulation-based technique for estimating density of secretive snakes that combines behavioral observations of snake road crossing behavior (crossing speed), effort-corrected road survey data, and simulations of spatial movement patterns derived from radiotelemetry, without relying on mark-recapture. Specifically, we use radiotelemetry data to parameterize individual-based movement models that estimate the frequency with which individual snakes cross roads and use information on survey vehicle speed and snake crossing speed to determine the probability of detecting a snake, given that it crosses the road transect during a survey. Snake encounter frequencies during systematic road surveys can then be interpreted in light of detection probabilities and simulation model results to estimate snake densities and to assess various factors likely to affect encounter rates.

We demonstrate the broad applicability of this approach through case studies of the imperiled southern hognose snake (*Heterodon simus*) in the North Carolina Sandhills and the invasive Burmese python (*Python molurus bivittatus*) in Everglades National Park, Florida. For both species, we use existing radiotelemetry and extensive road survey data to generate the first density estimates available for the species. We find that southern hognose exist at relatively low densities (0.17 per ha), raising concern that this species may not only have declined in geographic range but may also occur at relatively low densities and/or be declining in their strongholds, such as the North Carolina Sandhills. Our estimates of python density (1.5 – 5 per km²) provide baseline information that is critical for management of this harmful invader. Our results suggest that current control measures are insufficient to curb population growth and spread of pythons in South Florida, but highlight strong variation in python density over time. Overall, our method enhances our ability to study and manage many secretive snake species that are most effectively samples using road surveys. We discuss sensitivity of our results to assumptions and model parameters and provide guidelines for using our technique to estimate densities of secretive reptiles on DoD lands. Based on a recent inventory of amphibians and reptiles occurring on DoD lands, snake species are the most species rich taxa, represented by 131 species. Information on the density of these snake species on DoD lands is generally lacking.

I. Introduction

Knowledge of species distribution and abundance is a critical component of reasoned conservation and management decision-making. Both distribution and abundance estimation generally rely on a thorough understanding of detection probabilities. Without data on species' detection probabilities (i.e., the likelihood that *any individual* of a given species is detected in a given survey unit), the effort required to determine if a species occurs in a particular area is unknown and it is impossible to differentiate between true and false absences with statistical confidence. Likewise, knowledge of individual detection probabilities (i.e., the likelihood that *a specific individual* organism is detected in a given survey unit) is critical for estimation of species' abundances or densities. Several methods are traditionally used for estimating animal densities, including distance sampling, removal sampling, and most commonly, capture-mark-recapture techniques (CMR) (Rodda 2012). For species with extremely low detection probabilities or in situations where traditional techniques cannot be used, estimation of density is often impossible (Dorcas and Willson 2009). Unfortunately, species with low individual detection probabilities are often species that are of greatest conservation concern (Willson 2016).

Among reptiles, snakes are particularly secretive and the density and trends of most snake populations remain unknown (Parker and Plummer 1987, Dorcas and Willson 2009, Steen 2010, Todd et al. 2010). Recently, snakes have gained recognition as important components of vertebrate biodiversity and for the critical roles they play as predators and prey in many ecosystems (DeGregorio et al. 2014, Steen et al. 2014, Willson and Winne 2016). Thus, the need for effective snake conservation has become more apparent (Gibbons et al. 2000, Todd et al. 2010). Numerous snakes are now listed or proposed for listing under state or federal law and thus many stakeholders are required to consider snakes in their management plans. Although snakes can be abundant, many species are extraordinarily secretive and thus infrequently encountered (Dorcas and Willson 2009, Steen 2010). For example, recent experimental research has demonstrated that the detection rates for some snakes can be lower than 1%--that is, for every one snake found, investigators passed by more than 99 that remained undetected (Dorcas and Willson 2013). Without an understanding of individual detection probability, it is impossible to know whether low capture rates or failed surveys reflect true rarity, or simply secretive behavior. Thus, the secretiveness (or low detectability) of most snake species makes it extremely difficult to determine even if they are present at a particular site (Gibbons et al. 1997), let alone the size or density of the population (Steen 2010). Lack of density information limits our ability to assess the status or trends of most snake populations (Dorcas and Willson 2009).

Estimating densities or detection probabilities typically requires intensive CMR studies, which are not feasible for many snake species (Dorcas and Willson 2009, Willson 2016). However, many secretive snake species can be effectively captured using road surveys (Enge and Wood 2002, Willson 2016) and many are frequently studied using radiotelemetry (e.g., Steen and Smith 2009, Miller et al. 2012). Radiotelemetry studies provide considerable insight into the behavior and spatial ecology of secretive snake species (Kingsbury and Robinson 2016). Knowledge of movement patterns and behaviors gleaned from radiotelemetry studies can be used to parameterize individual-based models that simulate animal movement (Schwarzkopf and Alford 2002, Rupp and Rupp 2010). These types of individual-based movement models have been used to predict population responses to conservation actions, habitat loss, road effects, and to estimate the effects of landscape features on connectivity of

populations (Piou et al. 2007, Wang and Grimm 2007, Coulon et al. 2015, Allen et al. 2016, Heinrichs et al. 2016). A basic random-walk model defined by movement distance and turning angle distribution can be modified to incorporate behaviors such as home range maintenance or attraction to or avoidance of landscape features, including roads (Morales et al. 2004, McClintock et al. 2012). For example, Pauli et al. (2013) designed an individual-based model that modifies a basic random walk to incorporate multiple behavioral states between which individuals shift probabilistically based on factors such as life stage, past experiences, and the landscape through which they are moving. Random walk-based simulation models such as these provide an opportunity to predict the likelihood of a snake crossing a road, and thus a method for relating observation rates during road surveys to snake abundance.

Here, we develop a novel approach that integrates data from spatial ecology studies (i.e., radiotelemetric data) and road encounter data to estimate density of secretive snakes without needing to recapture individuals. We demonstrate the method using two case studies: 1) the imperiled southern hognose snake (*Heterodon simus*), one of the least understood snakes in North America; and 2) the Burmese python (*Python molurus bivittatus*), a damaging invasive species that is well established in South Florida and threatens numerous native species across much the southern United States. Our research provides the first science-based density estimates for these two species, setting a baseline for status assessment and management on DoD Lands. We discuss the assumptions and limitations of our approach as well as making recommendations for implementation of this method on DoD lands. Specifically, we address the following objectives:

Objectives:

1. Use simulation-based analysis of existing spatial movement data to calculate detection probabilities of snakes during systematic road surveys.
2. Apply knowledge of detection probabilities to determine densities of southern hognose snakes in the North Carolina Sandhills and invasive Burmese pythons in South Florida.

II. Density Estimation Approach

Our density estimation approach combines behavioral observations of road crossing behavior (crossing speed), effort-corrected road survey data, and simulation-based modeling of spatial movement to estimate population densities. Radiotelemetric data are collected to quantify movement metrics, including: frequency, distance, and direction of movement in relation to home range center and roads. These movement data are then used to parameterize individual-based movement models in a biased correlated random walk framework (Turchin 1998, Crone and Schultz 2008) to estimate the frequency with which individual snakes cross roads. Next, information on survey vehicle speed and snake crossing speed are used to determine the probability of detecting a snake, given that it crosses the road transect during a survey. Snake encounter frequencies during systematic road surveys are then interpreted in light of detection probabilities and simulation model results to estimate snake densities and to assess various factors likely to affect encounter rates.

Density estimation model

The first component of our density estimation approach uses information on vehicle speed and snake behavior to estimate snake detection probability on roads, thereby allowing counts during systematic road surveys to be corrected for imperfect detection, yielding unbiased estimates of true road crossing rate. In order to detect a snake that crosses the survey route during that survey, the surveyor's location and the snake's location must coincide in space. The distance the surveyor covers in the time it takes a snake to cross (Detection Distance; D_p [km]) is equal to the average snake crossing time (V_{snake} [min]) multiplied by the vehicle speed ($V_{vehicle}$ [km min⁻¹]; Eq.1).

$$(Eq. 1) D_p = V_{snake} \times V_{vehicle}$$

Thus, assuming that all snakes encountered by the survey vehicle are detected, the probability of detecting any individual snake that crosses during a survey (\hat{p}) is Detection Distance / Total Survey Distance (D_{survey} [km]; Eq.2).

$$(Eq. 2) \hat{p} = \frac{D_p}{D_{survey}}$$

Observed encounter rate (N_{obs} [snakes/h]) can be translated to an estimated total crossing rate (\hat{N}_{cross} [snakes km⁻¹ h⁻¹]) by dividing by \hat{p} and the total survey distance (Eq. 3).

$$(Eq. 3) \hat{N}_{cross} = \frac{N_{obs}}{\hat{p} \times D_{survey}}$$

Finally, estimated total crossing rate can be translated to density (\hat{N} [snakes km⁻²]) by dividing \hat{N}_{cross} by the estimated hourly crossing rate of individual snakes obtained from movement simulation models (ρ [crossings snake⁻¹ h⁻¹]; See below) and the width of the simulated landscape (A [km]; see below)(Eq. 4).

$$(Eq. 4) \hat{N} = \frac{\hat{N}_{cross}}{\rho \times A}$$

A closer examination of Equations 1-4 reveals that several terms cancel out when these equations are combined; yielding a greatly simplified overall equation for estimating density (Eq. 5).

$$(Eq. 5) \hat{N} = \frac{N_{obs}}{V_{snake} \times V_{vehicle} \times \rho \times A}$$

The resulting simplified formula (Eq. 5) can be thought of as dividing the observed encounter rate by the length of road that could be monitored with a 100% chance of detecting any snakes that cross (Detection Distance), and then dividing the result by the estimated hourly crossing rate of individual snakes.

Modeling Snake Movement

We developed a simple model to simulate snakes moving within a home range in order to estimate the daily probability that a snake will cross a road. Movement was modeled as a biased correlated random walk (BCRW) in continuous space with a wrapped Cauchy distribution of turning angles (Zollner and Lima 1999) and lognormal distribution of step sizes based on an analysis of the radiotelemetry data. The bias parameter was built into the model as the degree to which snakes choose a movement bearing according to the center of the home range and the bearing toward the road. The bearing that the animal chose at each time step was a weighted average of the bearing of the previous

step (plus random error drawn from the wrapped Cauchy distribution) and the bearing toward the home range center (or road), following:

$$\varphi_t = (1 - \beta)(\varphi_{t-1} + \gamma_t) + \beta\delta_t$$

Where φ_t is the bearing at time t , β is the strength of bias toward home range center or road, γ_t is the turning angle drawn from the wrapped Cauchy distribution at time t , and δ_t is the bearing toward the home range center at time t (Crone and Schultz 2008, Barton et al. 2009). Each individual was assigned a random home range center within a buffer (landscape) of width (A) surrounding a linear road bisecting a uniform landscape and was given an initial movement bearing from a uniform distribution (1-360 degrees). The parameter (A) is defined on a species-by-species basis, such that the model has a high likelihood of simulating all snakes with a chance to cross the road. However, the estimated density is generally insensitive to simulated landscape size. Increasing landscape size will decrease probability of detecting each individual snake, because snakes further from the road are less likely to cross. Thus, with a larger landscape, detection probability will be lower, resulting in a larger estimated population size, but we would then divide by a larger landscape, yielding the same estimate of density. Each time step was considered one day, and each simulation was run for 31 days. We calculated the proportion of snakes that crossed the road on the 31st time step of the simulation to estimate daily road crossing probability, and then divided by the diel activity period (hours per day when snake activity occurs) to calculate hourly individual road crossing probability (p). Because snakes' initial location in the model was always one meter away from their home range center, we ran the movement simulation for 30 time steps as a burn-in period to prevent initial location relative to home range center from influencing road crossing probability. Snake movement distances were randomly generated from a lognormal distribution and movement bearings were stochastic but also determined both by the location of the snakes' home range center and the location of a road. Behavior when roads were encountered was incorporated into the model as an additional bias in movement bearing.

III. Case Study 1: Southern Hognose Snake

The southern hognose snake (*Heterodon simus*; Fig. 1) is a fossorial species found in areas with well-drained sandy soils in the southeastern Coastal Plain from North Carolina to southern Mississippi. Over the last few decades, *H. simus* has declined or been extirpated throughout much of its historic range, and it has not been found in Alabama or Mississippi since the 1970s (Fig. 2) (Tuberville et al. 2000). *Heterodon simus* has been proposed for listing under the U.S. Endangered Species Act and is listed as threatened, endangered, or of special concern in every state where it occurs (or historically occurred), except Florida. It is found or potentially found on numerous military installations within the southeastern United States, including Fort Bragg (NC), Fort Gordon, Fort Stewart (GA), Fort Benning (GA), Eglin Airforce Base (FL), MCB Camp Lejeune (NC), and NAS Pensacola (FL). In states where it still occurs, its range has shrunk considerably (Tuberville et al. 2000, Gibbons and Dorcas 2005). Determining the current geographic range of *H. simus*, and the status of populations throughout its range, are critical for proper conservation and recovery management of the species. Unfortunately, because of the low

detectability of these secretive snakes, estimates of their population densities are entirely lacking. *Heterodon simus* are almost exclusively diurnal and are most frequently found during day-time road surveys from mid-September to early November (Enge and Wood 2002, Gibbons and Dorcas 2005, Beane et al. 2014). Substantial data exist on both their spatial ecology collected using radiotelemetry (J. Beane unpublished data, T. Tuberville unpublished data) and encounter rates on roads (Beane et al. 2014).

Methods

Road Crossing Speed

We gathered data on road crossing speed of *H. simus* through direct behavioral observations and videography of snakes encountered while naturally crossing roads. Specifically, we conducted diurnal road surveys for *H. simus* on low-traffic paved roads located on the Department of Energy's Savannah River Site, Barnwell Co., South Carolina, with particular effort during September–November, when *H. simus* are most frequently encountered (Enge and Wood 2002, Beane et al. 2014). When a snake was encountered, we stopped the vehicle at least 10 m away from the snake, shut off the engine, and observed the snake as unobtrusively as possible for the duration of the crossing event. In most cases, the event was videotaped using a Canon 7D digital camera or smartphone. Typically, the snake would freeze for a short period (usually < 1 min) when the vehicle first approached but then resume crossing the road using rectilinear locomotion, which is typical for this species when undisturbed. Once the snake resumed movement, the observer or video reviewer noted the time at which the snake's nose or tail tip crossed two known landmarks (e.g., center line, road edge, or obvious crack in pavement). Once the snake fully crossed, it was captured, measured (SVL, mass, sex), and released at its capture location. The distance traveled between landmarks was then measured (nearest cm), as well as the total width of the road. Any snake that turned around, froze for > 5 mins, or employed lateral undulation movement was considered disturbed and was not included in the dataset. In addition to our own observations, we queried other herpetologists in North Carolina and Florida who regularly encounter *H. simus*, recruited them to videotape snakes found naturally crossing roads using the methods described above, and analyzed the videos using the same methods. For each snake, crossing speed was calculated by dividing the distance traveled (cm) by the time elapsed (sec) and extrapolated to total crossing time, by multiplying speed by a typical road width of 550 cm (average width of road measured during behavioral observations and typical width of a paved two-lane road).

Road Surveys

We used an extensive existing and published database of systematic diurnal road surveys for *H. simus* conducted in the North Carolina Sandhills (predominantly in xeric uplands of Scotland and Moore counties) between 1996 and 2012 (Beane et al. 2014). Most roads surveyed bisected high-quality sandhill habitats, dominated by longleaf pine (*Pinus palustris*), scrub oaks (*Quercus* spp.), and wiregrass (*Aristida stricta*)—i.e., habitats historically preferred by *H. simus*—as well as sandy agricultural, residential, or other disturbed areas also heavily utilized by the species (Beane et al. 2014). We restricted analyses to the *H. simus* peak activity period of 1 Sept - 15 Nov and included nine years for

which survey effort was available (Table 1; Beane et al. 2014). The resulting dataset contained a total of 656 survey hours across 236 days (Table 1). Details of survey methodology are reported in Beane et al. (2014), but importantly an approximate driving speed of 48 km/h (30 mi per h; $V_{\text{vehicle}} = 0.8 \text{ km min}^{-1}$, was maintained and surveys were conducted on a haphazard, but ‘essentially random’ basis during the fall activity period (Beane et al. 2014). For this analysis we included only *H. simus* that were encountered alive (including living, but injured individuals; N = 12) during surveys, despite the fact that most individuals encountered were roadkilled.

Analysis of snake movement

We used data from 18 *H. simus* (11 males and 7 females) monitored using radiotelemetry in the Sandhills regions of North and South Carolina. In North Carolina 16 *H. simus* were tracked in the Sandhills Game Lands (SGL), and on some private lands, in Scotland County. The SGL represents a vast region (ca. 25,400 ha, not all contiguous) of state-owned sandhills habitat, managed for both game and nongame species by the North Carolina Wildlife Resources Commission. Sandhills Game Lands is managed with frequent prescribed fire (most tracts on 2-3 year burn rotation), and represents an apparent stronghold for *H. simus* in North Carolina. Radiotelemetry occurred from 1998 to 2011, and each snake was radiotracked for periods ranging from <2 months to >3 years, using mostly 5-gram transmitters with 12-month battery life (SB-2 and SB-2T, Holohil Systems, Ltd., Carp, Ontario), and a TRX-2000S receiver and 3-element Yagi antenna (Wildlife Materials, Murphysboro, IL).

In South Carolina, 2 snakes (1 male and 1 female) were tracked on the U.S. Department of Energy’s Savannah River Site, Aiken and Barnwell Counties. The SRS is an 800 km² government reserve in the Upper Coastal Plain of South Carolina. Prior to the establishment of the SRS in the 1950s, most of the land was under intensive agriculture with very little intact forest remaining except in the Savannah River floodplain (White and Gaines 2000). Although upland habitat on the SRS is currently managed for timber production, most areas are reforested and only 10% of the land has been developed for site operations. The animals included in this study occurred in the northeastern corner of the SRS in managed pine (primarily loblolly pine, *Pinus taeda*) plantations. Radiotelemetry occurred from 1998 to 2001, and each snake was radiotracked for 1 – 2 years using transmitters with a 12-month battery life (SB-2T, Holohil), 3 element-Yagi antenna, and a Telonics receiver. Snake locations were recorded (within 1 m) using GPS technology (Trimble Pro-XR, Sunnydale, CA).

To parameterize an individual-based movement model, we analyzed the radiotelemetry data from snakes radiotracked between 1 Sept and 15 Nov to match survey effort and the peak activity period for hognose snakes (Beane et al. 2014). For each snake, we estimated turning angle distribution (path straightness), mean net distance moved per day (step size), and response to roads using ArcGIS 10.1 (ESRI, Redlands, CA, USA). We additionally tested for the effects of sex on movement parameters (T-test). The time step of the simulation model consisted of one day; we therefore included only the movement path data that were collected on a daily basis for calculating and parameterizing mean step sizes and turning angle distributions. Therefore, for each snake, we calculated the mean daily movement distance (using only daily relocation data) and then we averaged the means across all snakes to generate the mean step size. Because the time scale of the simulation model and the time scale of the data were

equivalent, we did not discretize the data for estimation of movement parameters. We investigated response to roads using a Monte Carlo approach by generating random walk paths for each snake using empirical distributions of step size and turning angles (Shepard et al. 2008). We generated 1000 paths per snake, and for each simulated path, we calculated the number of times the snake crossed a road. We then generated an empirical distribution of number of crossing events from the simulations. Using an alpha of 0.05, we rejected the null hypothesis of no response to road (neither attraction or avoidance) if the observed number of road crossings fell into the upper or lower 2.5% of the frequency distribution of number of crossing events from the randomized paths.

Simulations

For *H. simus* movement simulations, each individual was assigned a random home range center within 500 m ($A = 1$ km) of a linear road bisecting a uniform landscape. This landscape size was selected to ensure that the model had a high likelihood of simulating all snakes with a chance to cross the road; using $A = 1$ km, snakes had a less than 0.005% chance of crossing a road from that distance if the snake moved directly toward the road. Each time step was considered one day, and each simulation was run for 31 days. We calculated the proportion of snakes that crossed the road on the 31st time step of the simulation to estimate daily road crossing probability, and then divided by 8 h (assuming all activity occurs between 9 am and 5 pm) to calculate hourly individual road crossing probability (p).

We simulated the movement of snakes under different movement scenarios. For each replicate simulation, we specified the following movement parameters: mean vector length (parameter defining turning angle distribution), strength of bias in response to road or home range center, and mean step size. Mean step size was a measure of the net distance a snake moved per day on average; this was parameterized using only daily relocations from the radiotelemetry data. The radiotelemetric data in our case study included limited numbers of road crossings, and thus we were unable to precisely parameterize the road bias component of our model. We therefore simulated a range of possible values for road bias, including both road avoidance and road attraction, and explored the sensitivity of our model output to assumptions about road behavior. The road bias parameter as defined in our model ranged from -1 to 1. A road bias value of 0 indicated that the snake biased its movement toward the home range center and displayed no behavioral response to the road. We considered this scenario our 'null' road bias scenario. A road bias value of 0.1 indicated that the snake biased its movement 10% toward the road and 90% toward the home range center. Similarly, a road bias value of -0.1 indicated that the snake biased its movement 10% away from the road and 90% toward the home range center (Examples of movement paths: Fig 3). The mean vector length was a measure of the straightness of a snake's movement path – a mean vector length of 0 indicates a fully random walk and a mean vector length of 1 indicates a completely straight movement path (100% probability of turning 0 degrees).

We explored the sensitivity of the model to road bias, turning angle distribution, and mean step size. We simulated a factorial set of plausible values for each of these parameters, including three levels of mean vector length (0.5, 0.7, and 0.9), five levels of road bias toward or away from a road (-0.3, -0.1, 0, 0.1, 0.3), and five levels of mean step size based on telemetry data (upper and lower range of step sizes, upper 95% confidence intervals (CI) and lower 95% CI of step sizes, and mean step size). Therefore, we simulated a total of 75 combinations of snake movement values. We simulated 35,000 snakes in

each treatment combination and calculated the percentage of 35,000 snakes that crossed a road as a measure of daily road crossing probability.

Results

Road crossing speed

We successfully timed nine wild *H. simus* encountered naturally crossing roads (Table 2), that did not exhibit behaviors indicating that they had been disturbed (prolonged freezing, turning, or movement via lateral undulation). These individuals represented a variety of snake sizes and included animals crossing both paved and unpaved (sand) roads. Crossing speed varied considerably among individuals (range = 0.6 – 2.9 cm/sec; mean = 1.5 cm/sec; Table 2), with no clear pattern relating to road type or snake size. Extrapolated to an average road width of 550 cm, we estimated that a snake would take an average of 7.69 min (95% CI = 5.42 – 9.96 min) to completely cross a typical road ($V_{snake} = 7.69$). Our crossing speeds were nearly identical to those of congeneric *H. platirhinos* measured in another study of snake road crossing behavior (Andrews and Gibbons 2005).

Encounter rate

A total of 656 h of systematic fall road surveys over 9 years in the North Carolina Sandhills yielded 54 captures of live *H. simus* (Table 1) and mean capture rate of 0.082 live snakes per hour of survey ($N_{obs} = 0.082$). Capture rates varied considerably among years (Table 1), but calculating an annual grand average across years yields a nearly identical capture rate of 0.083 (SD = 0.053) live snakes per hour of survey.

Movement modeling

Parameterization: We found that hognose snakes moved a mean of 16.9 m per day (SD = 13.17; Range: 3.61 m – 36.3 m among individuals) based on movement paths from 18 snakes. Step sizes were best fit with a lognormal distribution. We did not find evidence of road attraction or avoidance in any snake within the study because of a small number of relocations per snake path. We therefore were unable to detect road avoidance or road attraction.

Movement model: We found that the daily probability of crossing a road was affected by movement parameters. Across all parameter combinations, daily road crossing probabilities of individuals ranged from 0.035% to 2.4%. Mean step size, mean vector length, and attraction to road were positively correlated with probability of crossing a road. The parameter combination most closely approximated by the radiotelemetry data yielded a daily road crossing probability of 0.62%. For calculations of density (see below), we extrapolated this value to an hourly road crossing probability of 0.077% ($p = 0.00077$), assuming random movement throughout an 8 h daily activity period (9 am – 5 pm).

Density and model sensitivity

Inputting average values for road crossing speed, snake crossing time, and individual road crossing frequency into Eq. 5 yields a positive relationship between estimated density and encounter frequency during road surveys (Fig. 4). Based on this relationship and the average encounter rate during

road surveys of 0.082 snakes per h, the estimated density of *H. simus* in the North Carolina Sandhills is 17.14 snakes per km² or 0.17 snakes per hectare.

$$\hat{N} = \frac{0.082 \text{ snakes } h^{-1}}{7.69 \text{ min} \times 0.8 \text{ km min}^{-1} \times 0.00077 \text{ crossings snake}^{-1} h^{-1} \times 1 \text{ km}} = 17.14 \text{ snakes per km}^2$$

This density estimate is somewhat sensitive to various model parameters. For example, inputting upper and lower 95% confidence interval values for snake crossing speed (95% CI = 5.42 – 9.96 min) yields density estimates ranging from 0.13 – 0.24 snakes per hectare, with faster crossing speeds increasing estimated density (Fig. 4). Likewise, our density estimate varies with snake movement rate (step size) and behavioral response (attraction to or avoidance of) to the road (Fig. 5). Specifically, parameter values that reduce road-crossing frequency (smaller step sizes or stronger avoidance of the road) increase estimated density. Smaller step sizes yielded lower road crossing frequencies because snakes were less likely to move far enough away from their home range center to cross a road. Snakes were less likely to cross a road when road bias was smaller because negative road bias values corresponded to road avoidance (Fig. 5). Mean vector length was not a strong determinant of road crossing probability and therefore did not strongly impact expected density, likely because bias toward the home range center prevented high mean vector lengths from maximizing net displacement of snakes (thus increasing road crossing probability). Nevertheless, all movement parameter combinations within the 95% CI of step size and corresponding to a lack of strong behavioral avoidance of the road (<-0.2) yielded density estimates less than 0.5 snakes per hectare (Fig. 5). The lower range for the mean step size and the strongest road avoidance yielded the greatest density estimate (3.03 snakes/ha). Therefore, across all movement parameter combinations, estimated density ranged from 0.09 snakes/ha to 3.03 snakes/ha (9 – 303 per km²).

Conclusions – Southern Hognose Snake Density

Our approach provides the first density estimate for *H. simus* and provides an approach for generating baseline abundance data to inform effective conservation and management of secretive snakes for which density estimates were previously unobtainable. Using spatial movement parameters derived from radiotelemetry, mean observed road crossing speed, and encounter rate during road surveys, our analyses yielded a density estimate (including adults and juveniles) of 0.17 *H. simus* per ha in the North Carolina Sandhills. Based on our approach, it appears that *H. simus* occur at lower densities than many other terrestrial snakes (Parker and Plummer 1987). For example, densities based on CMR studies are between 1 and 10 per ha for both congeners, the eastern hognose snake (*H. platirhinos*) and the western hognose snake (*H. nasicus*; Platt 1969). Thus, low capture frequency of *H. simus*, even in suitable habitat, may be in part a result of low abundances. Low estimated density raises concern that *H. simus* may not only have declined in geographic range (Tuberville et al. 2000), but may also occur at relatively low densities and/or be declining in their strongholds, such as the North Carolina Sandhills. Several reasons have been proposed for the decline of *H. simus* including invasive fire ants and loss or fragmentation of the upland sandhill habitats they prefer (Tuberville et al. 2000). Our density estimate

provides a baseline for abundance of *H. simus* across a relatively large region, but care should be taken when extrapolating these densities to smaller land areas. In particular, our estimate should be viewed as representative of average density over the relatively large area covered by road surveys. Densities of *H. simus* certainly vary across landscapes, over time, and as a result of habitat quality (Enge and Wood 2002); thus, density estimates might vary considerably from our mean value (higher or lower, based on habitat quality) at smaller spatial scales. Future research should be conducted to determine how densities vary across the geographic range of *H. simus*, among various habitats, and temporally to evaluate population trends.

IV. Case Study II: Burmese Python

The Burmese python (*Python molurus bivittatus*; Fig. 6), one of the largest snakes in the world (to at least 6 m; Reed and Rodda 2009) and a long-time mainstay of the exotic reptile trade, has been introduced from its native range in Asia to South Florida (Snow et al. 2007b). Since recognition of their establishment in Everglades National Park (ENP) around the year 2000 (Meshaka et al. 2000), python numbers have increased dramatically (>2,000 removed since 2005) and the population has spread geographically to an area covering at least 8000 square kilometers and encompassing all of ENP and Big Cypress National Preserve (Dorcas and Willson 2011, Willson et al. 2011; Fig. 7). Pythons prey on a wide variety of mammals and birds including everything from rats to fully grown white-tailed deer (Snow et al. 2007a, Snow et al. 2007b, Reed and Rodda 2009, Rochford et al. 2010, Dove et al. 2011). They have already been implicated in severe declines of several once common mammals in ENP (marsh rabbits, raccoons, opossums, bobcats, etc.; (Dorcas et al. 2012, McCleery et al. 2015, Sovie et al. 2016, Reichert et al. 2017; Fig. 8). Known prey classified as Threatened or Endangered under the U.S. Endangered Species Act include the Key Largo Woodrat, and the Wood Stork (Reed and Rodda 2009, Dove et al. 2011). As pythons expand their range the impacts they will have on biodiversity of DoD lands and threatened and endangered species is of paramount concern. The U.S. Navy is the single largest landowner in the Florida keys. As pythons expand their range and negatively impact at-risk species, the regulatory burdens for managing those species is expected to shift disproportionately to DoD for recovering those same species. Finally, although fatal predatory attacks by pythons on humans have yet to be documented in South Florida, the potential for predatory attacks on humans by pythons is real and will only increase as pythons expand their range (Reed and Snow 2014).

Although invasive pythons are currently restricted to southern Florida, the geographic extent of the python invasion has increased substantially over the last decade and even the most conservative predictions show pythons potentially occupying the entire Florida peninsula and many areas of the Southeast and Gulf Coast (Rodda et al. 2011). At this time, the only DoD installation where pythons have been confirmed is Homestead AFB, but some climate models predict suitable environmental conditions for pythons throughout the southern United States, even as far north as Washington D.C., northern Texas, and central California (Fig. 9; Rodda et al. 2009). Naval Air Station Key West does not have pythons yet, but is at risk of them becoming a resident species with consummate effects on natural resources. Therefore, there is the potential for this invasive species to impact an estimated 130 military installations, ranges and training areas, since those same installations occur within the geographic area analyzed by Rodda et al. (2009)

Despite removal of large numbers of pythons in recent years, there are currently no science-based estimates of python abundance. This knowledge gap makes it impossible to evaluate the effectiveness of proposed control methods, design reasoned management initiatives, or understand or model python-prey interactions (Dorcas and Willson 2011). Recent research has indicated that individual detection probabilities of Burmese pythons are extremely low, even among snakes (Dorcas and Willson 2011). Dorcas and Willson (2011) conducted a controlled detectability study of pythons in a 31 x 25 m semi-natural enclosure housing ten pythons in South Carolina and found that detection rate was <1%. That is, even in a simplified environment with artificially high python density, searchers overlooked 99 pythons for every one detected. Low detectability implies that simple capture or removal rates of pythons in Florida are poor indicators of python abundance and effectively precludes application of CMR methods. Additionally, for invasive snakes such as the Burmese python, issues with very low detection probability are further complicated by the fact that release of captured individuals is potentially harmful to the environment. Fortunately, pythons are effectively captured using nocturnal road surveys and an extensive road database for pythons exist in the invasion epicenter, along the Main Park Road in Everglades National Park (Dorcas et al. 2012, Falk et al. 2016). Moreover, spatial movement of pythons in this area has been studied previously using radiotelemetry (Hart et al. 2015). Thus, Burmese pythons provide an ideal situation wherein knowledge of density is a critical conservation need and existing data allow implementation of our density estimation method with minimal additional data collection.

Methods

Road Crossing Speed

We gathered data on road crossing speed of pythons through direct behavioral observations and videography of pythons encountered while naturally crossing roads. Specifically, in 2013-2017, we conducted nocturnal road surveys for Burmese pythons in Everglades National Park. When a python was encountered, we stopped the vehicle at least 5 m away from the snake, shut off the engine and headlights, and observed the snake using indirect or red-filtered light as unobtrusively as possible for the duration of the crossing event. Typically, the snake would freeze for a short period (usually < 1 min) when the vehicle first approached but then resume crossing the road using rectilinear locomotion, which is typical for this species when undisturbed. Once the snake resumed movement, we noted the time at which the snake's nose or tail tip crossed two known landmarks (e.g., center line, road edge, or obvious crack in pavement). Once the snake fully crossed, it was captured and the distance traveled between landmarks was then measured (nearest cm). Any snake that turned around, froze for > 5 mins, or employed lateral undulation movement was considered disturbed and was excluded from the dataset. Following capture, snakes were returned to Everglades National Park, where they were generally euthanized and measured (SVL, total length) and sexed. For each snake, we calculated crossing velocity by dividing the distance traveled (cm) by the time elapsed (sec). We then extrapolated velocity to total crossing time for each snake by multiplying velocity by the total distance the snake would need to crawl while detectable on the road: 667 cm (average width of the Main Park Rd in ENP, measured at 10 locations spaced approximately evenly along the road) plus the total length of the snake (since the snake is visible on the road from the time its nose crosses the leading road edge, until its tail crosses the

opposite road edge). We examined relationships between snake size (total length) and velocity and length and total crossing time using linear regressions with $\alpha = 0.05$.

Road Surveys

We compiled an extensive database of systematic nocturnal road surveys for Burmese pythons conducted on the Main Park Road (MPR) in Everglades National Park between 2003 and 2017 (Table 4). The MPR is an asphalt road that runs ca. 65 km from the park entrance and Ernest F. Coe Visitor Center to the Flamingo marina on the coast of Florida Bay (Fig 7). Along this route, the road passes through habitats typical of ENP. The northeastern half of the route contains primarily freshwater sawgrass marsh, interrupted by periodic patches (islands) of rocky, higher, ground, vegetated with Slash Pine and Palmetto (Pine Rocklands). Along the middle section of the route, the road passes several ‘cypress domes’ and tropical hardwood hammocks that vegetate lower and higher ground, respectively. Freshwater marsh gradually gives way to saline glades and eventually dense mangrove forest for the final 18 km of the route. Surveys often included several short spur roads off the MPR, such as the roads to Mahogany Hammock, Pa-Hay-Okee, Royal Palm, and Research Road. In addition to our own road surveys, we included data collected by other university researchers studying pythons via road surveys (M. Miller, Auburn University; B. Smith, University of Florida), National Park Service Biologists (S. Snow), ‘authorized agent’ citizen-scientists (Falk et al. 2016), and an extensive daily road survey dataset from 2015 conducted by the USGS (Table 4). We restricted all analyses (road surveys and telemetry) to the period of 1 June – 30 November, corresponding to the period of greatest road captures (Falk et al. 2016) and when most python activity is nocturnal (Dorcas and Willson 2011). The resulting dataset contained a total of 2,009 survey hours and 89,493 km, over 542 nights (Table 4). Schedule of surveys varied among data sources, but many were systematic or pre-determined (i.e., every night, or every night during a trip; USGS, Willson), and most were spread across months from June to Nov. Given the large size of our dataset and relatively uniform weather of South Florida, we have no reason to suspect that survey schedule was biased with respect to snake activity. Most surveys were conducted starting at sunset and lasted several hours (mean survey length = 3.7 h in Willson dataset), maintaining an approximate driving speed of 48.3 km/h (30 mi per h; $V_{vehicle} = 0.8 \text{ km min}^{-1}$). In addition to a few long surveys that extended much of the night, we conducted some surveys from 01:00 to 05:00 h to examine diel patterns of python activity. We assessed diel variation in python road crossing activity using a subset of our dataset for which python capture times were available (Miller 2010, 2011, Willson 2009-2017; Table 4), by tallying the total number of hours surveyed during each hourly interval between 18:00 h and 05:00 h and dividing the number of python captures within each hourly by the resulting survey effort. For all analyses, we included only *P. molurus* that were encountered alive, but very few road-kills were found. For comparison of python relative abundance over time, we grouped surveys based on availability of data (Table 4) and important events, particularly a severe freeze in Jan 2010 (Mazzotti et al. 2011), into the following meaningful time periods: 2003-2005, 2006-2009, 2010-2013, 2014-2015, and 2016-2017; and examined pythons captured per hour of survey effort.

Analysis of snake movement

We used previously published data from 14 *P. molurus* (4 males and 10 females; 266 – 472 cm total length) monitored using radiotelemetry in the vicinity of the Main Park Road in Everglades National Park, Florida (Hart et al. 2015). Radiotelemetry occurred from 2006-2012, and each snake was radiotracked for periods ranging from 87-697 days (mean = 320 d), using mostly 5-gram transmitters with 12-month battery life (SB-2 and SB-2T, Holohil Systems, Ltd., Carp, Ontario), and a TRX-2000S receiver and 3-element Yagi antenna (Wildlife Materials, Murphysboro, IL).

To parameterize an individual-based movement model, we analyzed the radiotelemetry data from snakes radiotracked between 1 June and 30 Nov to match survey effort and the peak in nocturnal road crossing activity for snakes (Falk et al. 2016). For each snake, we estimated turning angle distribution (path straightness), mean net distance moved per day (step size), and response to roads using ArcGIS 10.1 (ESRI, Redlands, CA, USA,). We additionally tested for the effects of sex on movement parameters (T-test). The time step of the simulation model consisted of two days; we therefore included only the movement path data that were collected on a daily or two-day basis for calculating and parameterizing mean step sizes and turning angle distributions. Therefore, for each snake, we calculated the mean daily movement distance (using only daily or two-day relocation data) and then we averaged the means across all snakes to generate the mean step size. Because the time scale of the simulation model and the time scale of the data were equivalent, we did not discretize the data for estimation of movement parameters. We investigated response to roads using a Monte Carlo approach by generating random walk paths for each snake using empirical distributions of step size and turning angles (Shepard et al. 2008). We generated 1000 paths per snake, and for each simulated path, we calculated the number of times the snake crossed a road. We then generated an empirical distribution of number of crossing events from the simulations. Using an alpha of 0.05, we rejected the null hypothesis of no response to road (neither attraction or avoidance) if the observed number of road crossings fell into the upper or lower 2.5% of the frequency distribution of number of crossing events from the randomized paths.

Simulations

For *P. molurus* movement simulations, each individual was assigned a random home range center within 7 km ($A = 14$ km) of a linear road bisecting a uniform landscape. This landscape size was selected to ensure that the model had a high likelihood of simulating all snakes with a chance to cross the road; using $A = 14$ km, snakes had a less than 0.005% chance of crossing a road from that distance if the snake moved directly toward the road. Each time step was considered two days, and each simulation was run for 31 steps. We calculated the proportion of snakes that crossed the road on the 31st time step of the simulation to estimate probability over each two day step, and then divided by 18 h (assuming all road crossing activity occurs within an 9 h period each night) to calculate hourly individual road crossing probability (ρ).

We simulated the movement of snakes under different movement scenarios. For each replicate simulation, we specified the following movement parameters: mean vector length (parameter defining turning angle distribution), strength of bias in response to road or home range center, and mean step size. Mean step size was a measure of the net distance a snake moved per day on average; this was

parameterized using only daily relocations from the radiotelemetry data. The radiotelemetric data in our case study included limited numbers of road crossings, and thus we were unable to precisely parameterize the road bias component of our model. We therefore simulated a range of possible values for road bias, including both road avoidance and road attraction, and explored the sensitivity of our model output to assumptions about road behavior. The road bias parameter as defined in our model ranged from -0.3 to .3. A road bias value of 0 indicated that the snake biased its movement toward the home range center and displayed no behavioral response to the road. We considered this scenario our 'null' road bias scenario. A road bias value of 0.1 indicated that the snake biased its movement 10% toward the road and 90% toward the home range center. Similarly, a road bias value of -0.1 indicated that the snake biased its movement 10% away from the road and 90% toward the home range center. The mean vector length was a measure of the straightness of a snake's movement path – a mean vector length of 0 indicates a fully random walk and a mean vector length of 1 indicates a completely straight movement path (100% probability of turning 0 degrees).

We explored the sensitivity of the model to road bias and mean step size. We simulated a set of plausible values for each of these parameters, including three levels of road bias toward or away from a road (-0.3, -0.1, 0, 0.1, 0.3), and three levels of mean step size based on telemetry data (upper and lower 95% confidence intervals (CI and mean step size). We simulated 7,000 snakes in each treatment combination and calculated the percentage of 7,000 snakes that crossed a road as a measure of daily road crossing probability.

Results

Road crossing speed

We successfully timed 31 wild *P. molurus* encountered naturally crossing roads at night in ENP (Table 4), that did not exhibit behaviors indicating that they had been disturbed (prolonged freezing, turning, or movement via lateral undulation). These individuals represented a variety of snake sizes (62 – 283 cm total length) and both sexes. Crawling speed varied considerably among individuals (range = 0.9 – 7.9 cm/sec; mean = 3.3 cm/sec; Table 4). Extrapolated to an average road width of 667 cm and considering that a snake must also crawl its own body length to leave the road, we estimated that a snake would be detectable on the road (total crossing time) for an average of 5.28 min ($V_{snake} = 5.28$; 95% CI = 4.27 – 6.29 min). There was a positive correlation between snake length and crawling speed (linear regression; $p = 0.04$; $R^2 = 0.13$), but the low R^2 indicates that length accounts for little of the variation in speed. Moreover, there was not a significant relationship between snake length and total crossing time ($p = 0.21$; $R^2 = 0.05$). Thus, larger pythons crawled more quickly, but because they had to crawl a longer distance to get off the road, total crossing time was similar across snake sizes.

Encounter rate

A total of 2009 h of systematic road surveys over 14 years along the Main Park Road in Everglades National Park, Florida yielded 125 captures of live *P. molurus* (Table 4). However, effort-corrected encounter rates were highly variable over time (Fig. 10). Average python encounter rate was generally high (>0.1 per h) prior to 2010, and was particularly high (0.181 per h) in 2006-2009. However, this encounter rate was driven by one particularly successful dataset (Snow) in 2006 (Table 4). Following

a severe freeze that was known to have killed many pythons in the area in Jan 2010 (Mazzotti et al. 2010), mean capture rates were reduced substantially, to <0.08 per h. By 2016-2017, encounter rates had increased slightly, but were still fairly low ($N_{obs} = 0.076$ per h).

Diel distribution of road survey effort was not uniform across the night (Fig. 11); the majority of effort was expended between 20:00 and 01:00 h. To evaluate whether the biased distribution of survey effort might have biased our assessment of python road crossing rate, we examined effort-corrected encounter rate for each hourly period in a subset of our data for which python encounter times were available (Fig. 11). Pythons were seldom encountered before 21:00 and effort-corrected encounter rates were remarkably consistent between 21:00 and 04:00 h. Aside from the 0:300-0:400 h interval where no pythons were captured, probably due to chance and low effort, encounter rate only varied from 0.08 – 0.16 per h (Fig 11). Thus, our assumption of random movement across 9 h per night seems reasonable.

Movement modeling

Parameterization

We found that *P. molurus* moved a mean of 188 m per two day model step (SD = 186 m; Range: 13-991 m within individuals), based on movement paths from 14 snakes. Step sizes were best fit with a lognormal distribution. An analysis of the road crossing behavior of adult Burmese pythons failed to find evidence of road avoidance. Of the 13 pythons analyzed, 6 of these did not cross the road at all. However, this did not indicate road avoidance because random movement paths also failed to cross roads frequently (Table 1). Of the 7 pythons that did cross the road at least once, 2 of these showed evidence of road attraction (more observed crossings than predicted by chance; Table 5).

Movement model

We found that the probability of crossing a road was affected by movement parameters. Across all parameter combinations, two-day road crossing probabilities of individuals ranged from 0.5% to 2.2%. Mean step size and attraction to road were positively correlated with probability of crossing a road. The parameter combination most closely approximated by the radiotelemetry data (mean step size = 188 m; no road response to road) yielded a crossing probability of 1.1% per two-day step. For calculations of density (see below), we extrapolated this value to an hourly road crossing probability of 0.063% ($p = 0.00063$), assuming random movement throughout a 9 h daily activity period (8 pm – 5 am; the approximate period of darkness in early September, mid-way through our focal seasonal window of June - November).

Density and model sensitivity

Inputting average values for road crossing speed, snake crossing time, and individual road crossing frequency into Eq. 5 yields a positive relationship between estimated density and encounter frequency during road surveys (Fig. 12). Based on this relationship and the 2016-2017 average encounter rate during road surveys of 0.076 snakes per h, the estimated current density of *P. molurus* in the area around the Main Park Road in ENP is 2.05 per km² (0.02 per ha).

$$\hat{N} = \frac{0.076 \text{ snakes } h^{-1}}{5.28 \text{ min} \times 0.8 \text{ km } min^{-1} \times 0.00063 \text{ crossings snake}^{-1} h^{-1} \times 14 \text{ km}} = 2.05 \text{ snakes per km}^2$$

Based on the relationship derived above (Fig. 12), average crossing speed, and no behavioral response (attraction or avoidance) to the road, the density of pythons in ENP has varied substantially over time, from a peak of 4.88 per km² in 2006-2009 to a low of 1.31 per km² following the severe freeze in 2014-2015 (Fig. 12).

This density estimate is somewhat sensitive to various model parameters. For example, inputting upper and lower 95% confidence interval values for snake crossing speed (95% CI = 4.27 – 6.29 min) yields 2016-2017 density estimates ranging from 1.72 to 2.53 snakes per km², with faster crossing speeds increasing estimated density (Fig. 12). Likewise, our density estimate varies with snake movement rate (step size) and behavioral response (attraction to or avoidance of) to the road (Fig. 13). Specifically, parameter values that reduce road-crossing frequency (smaller step sizes or stronger avoidance of the road) increase estimated density. Smaller step sizes yielded lower road crossing frequencies because snakes were less likely to move far enough away from their home range center to cross a road. Snakes were less likely to cross a road when road bias was smaller because negative road bias values corresponded to road avoidance (Fig. 13). Nevertheless, all movement parameter combinations within the 95% CI of step size and corresponding to a lack of strong behavioral attraction to or avoidance of the road (attraction -0.1 to 0.1) yielded density estimates ranging from 1.05 – 4.50 pythons per km² (0.01 – 0.45 per ha) at the 2016-2017 encounter rate of 0.076 per hour (Fig. 13).

Conclusions – Burmese Python Density

We estimate that density of *P. molurus* in the area around the Main Park Road (MPR) in Everglades National Park (ENP), Florida has varied from approximately 1.5 – 5 per km² over the past decade, with a current (2016-2017) average density of 2.05 per km². This region represents the historical core of the python's invasive range (Dorcas and Willson 2011) and contains habitats broadly representative of ENP – extensive freshwater and brackish marsh, mangrove forests, interspersed with pockets of hardwood hammock and pine rockland. If densities along the MPR are representative of ENP, then extrapolating to the extent of ENP (3,988 km² of non-open water habitat) yields an approximate current population size of 8,000 pythons within the park and as many as 20,000 at peak abundance in 2016-2009. Extrapolations beyond the National Park are risky, because of dramatic differences in habitat and likely variation in density related to the progression of the invasion, but our results certainly suggest that there are tens of thousands of pythons across the >10,000 km² known area of invasion in South Florida. Our results are relatively robust to variation in model parameters, as evidenced by sensitivity analyses across the range of uncertainty in model parameters, which resulted in density estimates ranging from approximately 1 to 5 pythons per km². Although our method is subject to a variety of assumptions, our results provide a first step in filling a critical knowledge gap in our understanding of Burmese python biology and are a foundation for future efforts to manage and study this damaging invasive species.

Even though the habitat surrounding the MPR is broadly representative of the overall habitat in ENP, there are still potential factors that may have biased our assessments of density. Potential sources of bias are listed in detail in Section V (below), but particularly germane to pythons is the fact that pythons have been collected along MPR for over a decade. Between 2002 and 2014, over 600 pythons have been captured along the MPR and permanently removed (Falk 2016). Thus, the python population around the MPR may be reduced in comparison to the overall landscape. In addition, this collection presumably selectively removes individuals that have home ranges or behavioral patterns that predispose them to crossing roads, potentially artificially lowering road encounter frequency. Also, on any given night there is usually at least one vehicle (in addition to our own) searching for pythons along the MPR and it is possible that other collectors might have removed snakes that we would otherwise have encountered by our surveys. Finally, our assessment of python movement patterns may have been influenced by the fact that most of the telemetered pythons were adults and that much of the telemetry was conducted via aircraft (helicopter or fixed-wing plane). Adult snakes frequently move more extensively than juveniles (Jellen and Kowalski 2007) and low precision of aerial relocations (Hart et al. 2015) might cause us to overestimate movement rates. All of these factors would lead to underestimates of python density.

No previous studies have rigorously estimated density of *P. molurus* or closely related species, but our results agree reasonably well with what little data are available on python density. In the only published in-depth ecological study of *P. molurus* in its native range (India; Bhupathy and Vijayan 1989) recorded a maximum of 144 and 111 individual pythons within a 29 km² wildlife refuge over two winters, respectively, yielding an approximate density of 5 per km². Although this estimate may have been biased by double-counting of individuals that switched dens and/or low detectability of some individuals, especially juveniles (Bhupathy and Vijayan 1989), it is noteworthy that our estimate is similar to the available data from the native range of *P. molurus*. On three occasions, agricultural activities have serendipitously allowed for enumeration of pythons in quantified areas in South Florida. In 2006, 44 dead pythons were found following disking/plowing of a 607 ha section of fallow agricultural land along the eastern border of ENP. An 81 ha section of this same area was disked in 2009 and researchers carefully monitoring the equipment found 4 dead/moribund pythons, as well as 11 live individuals that would presumably have survived the event and remained undetected (Reed et al. 2011). Thus, assuming at 64% of individuals in 2006 escaped detection, the disked areas contained approximately 14.9 and 13.6 pythons per km², in 2006 and 2009, respectively. These densities are higher than our estimated densities from this study, but this is perhaps unsurprising, given that these fallow agricultural fields contained abundant (perhaps unnaturally so) rodent prey (Reed et al. 2011) and that these events occurred prior to the 2010 freeze that coincided with a decline in python numbers within the southern portions of ENP (Fig. 10; Mazzotti et al. 2011, Falk et al. 2016, Mazzotti et al. 2016).

Our results provide a critical baseline for management of invasive pythons in Florida. Up till now, it has been impossible to gauge or model the efficacy of python removal initiatives because the potential pool of undetected snakes was unknown. Our density estimates are sufficiently low that removal of moderate numbers of pythons (10s to 100s) from relatively small areas has the potential to impact populations. However, it should be noted that suppression over large areas will necessitate removal of hundreds to thousands of individuals, which is probably infeasible with current capture

technologies. Likewise, efforts that spread captures over large areas are unlikely to have an appreciable effect on the overall population. Finally, our models highlight the high movement potential of these snakes. In fact, it is noteworthy that the hourly individual road crossing probability of *P. molurus* (0.00063 at mean parameter values and assuming no road response) was similar to that of *H. simus* (0.00077). Yet, estimated density of pythons was considerably lower because the high movement potential of pythons meant that the road was effectively sampling a much larger portion of the surrounding landscape. Even if standing density of *P. molurus* at any location is low, large home range size (average = 22.5 km²; Hart et al. 2015) means that many individuals may use a particular area and suggests that animals may rapidly recolonize an area where management has successfully suppressed the local population.

It is also important to note that our density estimate represents an average across the area sampled and density is almost certainly not uniform across the landscape. The Main Park Road in ENP is a long transect that passes through many of the major habitats representative of ENP (Falk et al. 2016), all of which are used by *P. molurus* to some degree (Hart et al. 2015). However, our current dataset is not able to examine variation in abundance among habitats, and it is likely that some habitats harbor substantially higher densities than our overall average. In addition, there are almost certainly hotspots that harbor extremely high densities and potentially draw animals from a large footprint of the surrounding landscape. Removal data suggest that canal levees may represent such hotspots, but further research is needed to determine if whether high capture rates on canal levees represent unusually high standing abundance in those habitats, congregation of snakes in those habitats from a large footprint of the landscape, high detectability of snakes using those habitats, or a combination of these factors. Finally, our dataset highlights the potential for dramatic variation in abundance of pythons over time. Assuming consistent movement patterns (i.e., individual road crossing probability) our analyses suggest that python density along the Main Park Road has varied from 1.5 to 5 per km² over a decade (Fig 10). The most dramatic change in abundance coincided with an extreme cold event in winter 2010 that is documented to have killed many pythons in South Florida, including 9 of 10 telemetered adult pythons located within our study area (Mazzotti et al. 2010). Other researchers have documented a reduction in capture rates of pythons during road surveys of the Main Park Road (Mazzotti et al. 2016; Falk et al. 2016), as well as a shift in the spatial distribution of removals from the MPR to other portions of the park (Falk et al. 2016). This shift might be attributable to higher survival of pythons in artificial habitats (e.g., canal levees; Mazzotti et al. 2010) and may suggest that areas away from the MPR did not experience as severe reduction in abundance during the freeze. Our density estimation method provides a tool for consistent long-term monitoring of python abundance both within ENP and in other areas of South Florida. Such initiatives should include comparisons of movement (i.e., telemetry in different habitats, regions, and over time).

Our density estimate for Burmese pythons in ENP also has implications for understanding the impacts of this invasive species on native wildlife. Pythons prey on over 40 native species (Snow et al. 2007a, Snow et al. 2007b, Reed and Rodda 2009, Rochford et al. 2010, Dove et al. 2011), and they have been linked to severe declines of a wide range of mammals and apparent local extirpation of marsh rabbits through both spatial-temporal correlations of decline (Dorcas et al. 2012, Sovie et al. 2016, Reichert et al. 2017) and experimental marsh rabbit translocations that documented pythons as the

primary rabbit predators in ENP (McCleery et al. 2015). The ability of pythons to suppress and even extirpate mammal populations in South Florida is particularly surprising because these prey share a co-evolutionary history with a diverse native predator community that includes large rabbit eating snakes, such as the eastern diamondback rattlesnake (*Crotalus adamanteus*). The mechanisms driving the severe impacts of pythons on mammals are not well understood but are critical to understanding this damaging invader. Although we estimate that ENP is home to a large python population, densities are not particularly high. In fact, large native snake species commonly attain densities greater than one per hectare (100 per km²; Parker and Plummer 1987), more than an order of magnitude higher than our density estimate for pythons in ENP. Even *C. adamanteus* has been estimated to occur at densities as high as 1.32 or 2.77 per ha (132, 277 per km²) in suitable habitat (Means 2017) and is common in some areas of ENP. Thus, high density is almost certainly not the only factor, and may not even be the primary factor, driving impacts of pythons on mammals. It is likely that more subtle aspects of foraging ecology, energetics, or predator-prey behavioral interactions are critically important. By providing the first estimates of python density this study provides a foundation for modeling python-prey interactions and, when coupled with studies of energetics and digestive physiology, estimating rates of prey consumption by pythons in the field.

V. Considerations and Assumptions

We have developed and demonstrated an approach for estimating abundance based on likelihood of detection during road surveys. Our method is independent of traditional CMR approaches and thus shows particular promise for estimating abundance of species that have inherently low individual detection probabilities, such as many snakes. Ultimately, our method should be validated through direct comparison with CMR in a species amenable to both methods. Initially, however, we demonstrate the potential of our method using case studies of the southern hognose snake (*H. simus*) and Burmese python (*Python molurus bivittatus*). We chose these species because: 1) they are excellent examples of secretive snake species that are most effectively sampled using road surveys; 2) both species had substantial existing datasets examining spatial movement (radiotelemetry) and road encounter frequency; and 3) both are species of considerable management or conservation concern.

Although our approach shows promise for providing the first information on density of many secretive and rare snake species, it is not without assumptions and limitations. Below we discuss the important assumptions underlying our density estimation methods, provide commentary on those assumptions, and recommend steps that may be taken to minimize violation of assumptions and maximize accuracy and precision of density estimates. Finally, we make specific recommendations for implementing our density estimation method to study secretive reptiles on DoD lands.

Assumptions

Assumption 1: Densities of snakes near roads are representative of the area of interest.

Because our density estimation method relies on abundance data collected using road surveys, extrapolation of density estimates generated using this method relies on assumptions about how representative roadside habitats are of the overall landscape. Without additional data comparing abundance or movement of snakes relative to roads, the implicit assumption is that roadside habitats

harbor similar snake densities as habitats that do not border roads. This assumption is unlikely to be completely valid under most situations, but the implications of making this assumption will vary based on the biology of the species and characteristics of the landscape and roads. For example, for many species, roadside habitats are likely to harbor reduced snake densities due to road mortality and/or behavioral avoidance of roads or roadside habitats (e.g., Robson and Blouin-Demers 2013). Alternatively, species that prefer edge habitats or high ground provided by road beds in low lying areas may actually be concentrated in roadside habitats, leading to density estimates that are elevated relative to the overall landscape.

Despite these potential sources of bias, there are reasons to believe that assuming snake densities near roads are representative of the overall landscape may not be totally unwarranted. In many regions of the world, road densities are so high that a large proportion of the total land area is relatively close to a road. For example, a study in 2003 found that over 80% of the land area of the U.S. was within 1 km of a road (Riitters and Wickham 2003). Furthermore, although numerous authors have documented large numbers of road-killed snakes and expressed concern that road mortality negatively affects snake populations, little quantitative data exist demonstrating that snake population densities are depressed near roads. For example, Patrick and Gibbs (2009) deployed coverboard arrays systematically at different distances from roads across three sites, but found no relationship between snake abundances and distance to roads. Likewise, intensive road surveys of snakes along a transect in California in the 1970s and 1990s revealed a dramatic increase in relative abundance of one species and little change in relative abundances of the remaining nine species over time, despite a substantial increase in traffic volume (Sullivan 2000). Thus, although future research will clearly be needed to evaluate the implications of road effects on density estimates of various snake species, there is currently little evidence that density estimates near roads would be strongly biased.

Assumption 2: Timing of road surveys is unbiased relative to snake movements.

Because telemetry typically does not provide fine-scale timing of snake movements, our current models assume that all crossing events are detectable by surveyors and that surveys are not biased towards periods of high or low road crossing activity. The best approach to avoid violations of this assumption is to define a diel activity window expected to contain most or all activity and then conduct road surveys on a systematic or randomized schedule relative to that activity window. Most importantly, researchers must avoid conducting surveys only during expected periods of greatest activity (i.e., cherry-picking times of day or weather conditions when captures are expected to be greatest). If survey effort is not systematic or randomized, researchers would do well to test for diel and/or weather related variation in road encounter rates (as we have done for *P. molurus*) and possibly adjust conditional capture probability if there is evidence that road crossing rates are biased relative to search effort. Finally, larger datasets will tend to average out chance variation in encounter rates and yield more accurate and precise mean parameter values. Thus, we recommend collecting extensive road survey datasets to maximize precision and minimize bias in density estimates.

Assumption 3: Movement models accurately simulate snake movements and road crossing frequencies.

Movement models are never going to perfectly replicate snake movement paths; the main concern relative to our method is that we accurately predict road crossing frequency. There are several

factors that could potentially affect our ability to accurately estimate road crossing frequency with movement models. First, it is important that models are constructed at the temporal scale at which animals are likely to make significant movements, and that the temporal scale of empirical movement data (i.e., telemetry data) match that scale. If the model is constructed at coarser temporal scales, or if snakes move extensively between relocations (i.e., movements are missed because tracking is too infrequent), the model may underestimate road crossing probability and therefore overestimate density. Many snakes make long-distance movements relatively infrequently, making a one-day time step appropriate for many species. However, finer scale movement data, either through more frequent relocations or technologies that allow for automated relocation (e.g., satellite tracking or automated telemetry) or continuous tracking of movement paths (e.g., threadpooling or powder tracking; Tozetti and Martins 2007, Furman et al. 2011) could provide insight into the appropriate temporal scale for the model (Ward et al. 2013). Ideally, the accuracy of movement models should be examined by testing estimated crossing frequencies and emergent spatial movement metrics (e.g., home range size) against the empirical telemetry data.

Second, it is important that empirical movement data be representative of the snake population. For that reason, telemetry should ideally include both sexes and reproductive and non-reproductive individuals. Unfortunately, due to constraints of transmitter size, it is sometimes not possible to track juvenile snakes, which often move less extensively than adults (e.g., Jellen and Kowalski 2007). In this case, researchers should consider that basing models on movement data from adults could lead to overestimation of road crossing frequency and thus underestimation of abundance. An ultra-conservative approach to avoiding this type of bias would be to exclude juveniles from the road dataset entirely and stipulate that the density estimate only applies to the adult component of the population. Continued miniaturization of radio transmitters alternative technologies such as PIT tag telemetry (Oldham et al. 2016) and powder tracking (Furman et al. 2011) will undoubtedly improve the ability of researchers to monitor movements of a wider range of individuals at finer temporal and spatial scales in the future.

Third, our models are particularly sensitive to assumptions about road bias. A clear understanding of how roads impact the movement decisions (e.g, Andrews and Gibbons 2005) of animals is critical to the implementation of this modeling approach, especially in situations where animals might display strong road avoidance. Relatively few studies have rigorously addressed how roads influence snake behavior or snake spatial movement (but see Andrews and Gibbons 2005, Robson and Blouin-Demers 2013, Siers et al. 2014). Neither of our case studies found strong evidence for behavioral responses of snakes to roads, but our datasets (especially *H. simus*) were limited by having relatively few individuals that encountered roads while being monitored via radiotelemetry. Robson & Blouin-Demers (2013) found evidence that *H. platirhinos* behaviorally avoided paved roads but did not avoid unpaved roads. Therefore, snakes' reactions to roads may be context-dependent, necessitating further research into the mechanisms or cues underlying snakes' interactions with roads.

Finally, our movement models currently use a simple random-walk framework and ignore much potential variation in movements due to spatial complexity of the landscape and/or orientation of animals towards habitat features or each other. However, our modeling framework could easily be adapted to incorporate additional complexity into the abundance estimation process. For example, our approach makes the assumption that habitat surrounding roads is homogenous and that snakes are

randomly distributed throughout the landscape. Longer term and finer temporal scale radiotelemetry studies could provide the information needed to create spatially-explicit models in which habitat type and species' movement behavior vary throughout the landscape or in response to conspecifics (clumped or uniform distribution of home ranges, for example). However, researchers should remember that complexity incurs compounding error and complexity should only be favored when there is evidence that it is alleviating imprecision or bias in the density estimation process.

Assumption 4: All snakes encountered by the survey vehicle are detected.

Our current model assumes that all snakes encountered by a survey vehicle are detected. If some snakes that are encountered by the survey vehicle are missed by observers, road crossing frequency estimates could be negatively biased, leading to underestimation of density. This assumption is probably reasonable for our case studies since surveys were conducted on paved roads during the day at low speed (*H. simus*) or were targeting species large enough that even juveniles are difficult to miss (*P. molurus*). However, under more challenging viewing conditions (e.g., night, higher speed, smaller species, or unpaved road), detection probability might need to be adjusted to account for imperfect detection. Surveys should attempt to maximize detection by maintaining a consistent slow speed and ideally having a second observer in the vehicle. In situations where crossing snakes may be overlooked, researchers could deploy clay models or road-killed snakes at random locations, without knowledge of the individual conducting the survey, to quantify probability of detection. In this case, it would be relatively easy to adjust conditional detection probability in models to account for imperfect detection.

Bias in detection rates during surveys could also result from factors that prevent animals that should be detected alive from being encountered alive. For example, removal of snakes crossing roads by other snake hunters could lead us to underestimate road encounter frequency, and thus, density. Additionally, because it was not possible to determine if a road-killed individual would have been detected crossing naturally had it not been hit, we were forced to exclude all road-killed snakes in our case studies. It is possible that some of these snakes would have otherwise been detected alive. Neither of these sources of negative bias in road encounter data have been quantified and they are not currently accounted for in our models. An extension of our approach that would allow researchers to take advantage of roadkill data would be to use data on traffic volumes and crossing speed to calculate the probability that a snake would be hit while crossing (e.g., Andrews and Gibbons 2005) and thus be detectable as roadkill. We did not have the data necessary to attempt this with either of our datasets, but doing so would greatly increase the number of road detections. For example, the vast majority (643 of 764) of *H. simus* detected by Beane et al. (2014) were dead.

Assumption 5: Road crossing speeds are unbiased.

Our models rely on the assumption that we have accurate measures of the time it takes snakes to cross roads. Road crossing speed can be influenced by a variety of factors including environmental conditions (temperature, sun or moonlight, rain, etc.), characteristics of the road (width, degree of canopy cover, road material, traffic volume, etc.; Andrews and Gibbons 2005), and whether or not the snake is disturbed while crossing. We have greatly minimized potential bias due to these factors by measuring speeds of naturally crossing snakes, usually on the same roads where we were attempting to

estimate density. This approach ensures that snakes are crossing under conditions that are typical of movement and by attempting to time every snake encountered, we assume that the variation in crossing times we observed was representative of natural road crossings. Additionally, we were diligent in our attempts to disturb crossing snakes as little as possible while timing their speed (e.g., stopping well away from the snake, shutting of the vehicle to prevent vibration, maintaining a low profile) and excluding any animals that showed obvious signs of being disturbed by our presence (e.g., prolonged immobility, turning, use of lateral undulation movement, rattling tail). One final source of bias that was not important in our case studies was potential variation due to road surface. In particular, although we focused exclusively on paved roads, it is likely that road crossing behavior and speed would differ between paved and unpaved road surfaces. We strongly suggest that researchers attempting to use our method take a similar approach and consider important sources of variation and bias in measurements of road crossing speed, including type of road surface. Finally, it is worth mentioning that the most unbiased way to measure crossing speed would be to record naturally crossing snakes using remote technology (i.e., remote cameras). Although not possible in our case due to rarity of the study species, this approach might be possible under other circumstances.

Recommendations for Implementation on DoD Lands

Our case studies demonstrate the applicability of our road-based density estimation method for secretive and/or rare snake species for which traditional CMR studies are infeasible. Our approach shows strong promise for understanding the status, conservation, and management of a variety of species, and in some cases could be used to generate rough approximations of density using existing data sources. This is important not only for managing snake species and populations, but is especially important for managing at-risk snakes. Resulting data could be utilized to assist with regulatory consultations, such as section 7 consultations under the Endangered Species Act (Endangered Species Act (16 U.S.C. 1531-1544, 87 Stat. 884) and estimating “Take” of training and testing missions that may affect listed species. With the aforementioned assumptions in mind, we conclude with specific recommendations for implementing this method on DoD Lands. Specifically, we discuss species and landscapes that are most amenable to the technique and considerations for data collection and analysis.

Focal Species:

Although our density estimation method provides an opportunity to understand populations of a variety of secretive or rare snakes, some species conform more readily to the assumptions listed above than others, and characteristics of the species must be considered carefully before implementing our method. In Table 6, we list snake species documented on DoD lands (based on the DoD PARC Herpetofauna Database), biological characteristics that affect their suitability for our road based density estimation method, and rank them in terms of suitability. Perhaps most obviously, our method is only suitable for species that are effectively sampled using road surveys and those that are large enough to study via radio telemetry or other methods of monitoring spatial movement. Additionally, because temporal scale of spatial movement data must be sufficiently fine to capture road crossing movements, our method is most suitable for relatively sedentary species. For species that make frequent and/or extensive non-linear movements (e.g., racers, coachwhips, and other active species) it is likely that road

crossing frequency would be substantially underestimated by models based on daily relocations. Indeed, for such species, it may be nearly impossible to monitor fine-scale movements or to time natural road crossing velocity without influencing the snake's behavior. Thus, currently, our method is likely most appropriate for species that make linear long-distance movements relatively infrequently, such as those that use ambush foraging or those that are primarily fossorial. However, it is likely that future technological advances, especially GPS/satellite and automated telemetry, will greatly increase the pool of species amenable to our movement modeling approach, as well as allowing quantitative assessment of the optimal temporal scale for movement models. Variation in movement and road encounter frequency data (and thus imprecision of abundance estimates) can be minimized by focusing on species with distinct seasonal and diel activity patterns, as was the case for *H. simus*. Finally, our case studies demonstrate the ability of our method to take advantages of existing datasets to estimate density with minimal collection of original empirical data. Thus, managers might particularly consider species that have already been studied via radio-telemetry and/or road surveys. However, in this case, particular attention should be paid to potential bias incurred by violations of assumptions 2 & 3, since data were not collected with our modeling approach in mind.

A diverse array of North American snake species, some of which are of conservation concern, conform well to the criteria listed above and are excellent candidates for our density estimation approach (Table 6). Perhaps most obvious are vipers (i.e., rattlesnakes, copperhead, cottonmouth), which are generally ambush foragers that make infrequent long-distance movements, especially during the breeding season. Other good candidates include many of the larger terrestrial and/or fossorial species such as gopher and pinesnakes (*Pituophis* spp.), kingsnakes and their relatives (*Lampropeltis* spp.), hognose snakes (*Heterodon* spp.), and ratsnakes (*Pantherophis* spp.). Even some fossorial lizards such as the beaded (*Heloderma* spp.) and legless lizards (*Ophisaurus* spp.) and terrestrial turtles (e.g., box turtles, *Terrapene* spp.) may be candidates for our approach. For many of the aforementioned species, road surveys are the most effective standardized survey method (Willson 2016) and some have been studied previously on DoD lands.

Focal Landscapes:

Our method is based on a random walk and currently the only sources of movement bias are the road and past movement (i.e., orientation towards a home range center). Although our movement models can be expanded to incorporate additional spatial complexity, our method is currently most applicable to relatively uniform landscapes that contain long road transects that pass through natural habitats. Following Assumption 1, it is particularly important that roadside habitats be as representative of the overall landscape as possible. Thus, researchers should avoid situations where land use near roads is different from the surrounding landscape (e.g., agricultural or residential development along roads). Finally, ideal study sites will have well-maintained roads with low traffic to minimize loss of detections to collection and roadkill and maximize safety of surveyors. Fortunately, many military installations meet all of these criteria and thus provide ideal landscapes for implementation of this technique. As with any work on active installations, researchers should be sure that field activities do not disrupt base operations and that appropriate safety precautions are taken when in the field.

Road Surveys:

To avoid violations of Assumption 2 (Timing of road surveys is unbiased relative to snake movements), it is important to ensure that road surveys are unbiased relative to snake movements. The ideal way to meet this assumption is to pre-define conditions (season, time of day, weather conditions) that are suitable for snake movement and then conduct road surveys on a randomized or systematic basis throughout those pre-defined activity windows. A critical point is that surveys not be concentrated on particular or 'best' conditions, unless enough data on activity exist that capture probabilities could be adjusted to account for this source of bias. Also for this reason, it may be best to constrain data collection and analyses to relatively short seasons when road crossing movements are expected to peak, as we have done for *H. simus*. Finally, a large volume of road survey data should be collected to average out chance variation due to environmental conditions and other factors.

In order to meet Assumption 4 (All snakes encountered by the survey vehicle are detected), which deals with imperfect detection of snakes on roads, researchers should take steps to maximize detectability, such as using only trained observers, maintaining a speed that allows for careful monitoring of the road, and having a second observer in the vehicle. In cases where imperfect detection seems likely (small species, challenging viewing conditions, etc.), researchers could conduct experiments to quantify detection rates and incorporate empirical estimates of detection probability into calculations, as outlined above under Assumption 4.

Telemetry and Movement Modeling:

Given that our approach is critically dependent on the ability of movement models to accurately predict road crossing frequency (Assumption 3), we suggest collecting movement (i.e., radiotelemetry) data at the same location where road surveys are occurring and including all representative demographic groups (i.e., both sexes, different ages). Telemetry data should coincide with the seasonality of road collection data and the temporal scale of telemetry data collection should be appropriate to the expected movements of the species (daily for most species, but perhaps more frequently for species that move extensively). Likewise, given that our results are sensitive to responses of snakes to roads (avoidance or attraction), researchers should attempt to track animals near roads, allowing for quantitative evaluation of road response behaviors. These studies should also consider potential differences between paved and unpaved roads, as snakes have been shown to respond differently to different road surfaces in previous research (Robson & Blouin-Demers 2013).

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Publications Associated with Project

- Willson, J. D., R. W. Snow, R. N. Reed, and M. E. Dorcas. 2014. *Python molurus bivittatus* (Burmese python). Minimum size at maturity. *Herpetological Review* 45:343-344.
- Willson, J.D., S. Pittman, J. Beane, and T. Tuberville. In review. A Novel Approach for Estimating Densities of Secretive Snakes Based on Road Survey and Spatial Movement Data. *Wildlife Research*.
- Willson, J.D., S. Pittman, M. Miller, B. Falk, B. Smith, C. Romagosa, C. Petersen, R. Lovich, and C. Hart. In preparation. Density of invasive Burmese pythons in Everglades National Park. Expected Submission: Dec 2017.

Professional Presentations Associated with Project

- 2017. Willson, J.D. and S. Pittman. A novel approach to estimating density of invasive Burmese pythons in South Florida. Joint Meeting of Ichthyologists and Herpetologists. Austin, TX.
- 2016. Willson, J.D. Biology and Impacts of Invasive Burmese Pythons in South Florida. Department of Biology, Missouri State University. Invited departmental seminar.
- 2016. Willson, J.D., S. Pittman, T. Tuberville, J. Beane, and M. Dorcas. A Novel Technique for Estimating Density of Secretive Upland Snakes. Joint Meeting of Ichthyologists and Herpetologists. Joint Meeting of Ichthyologists and Herpetologists. New Orleans, LA. July 2016
- 2013. J.D. Willson. Density and Detection of Invasive Constrictors: Challenges and Opportunities. DOI Interagency Invasive Constrictor Technical Meeting. Everglades National Park, FL. (Oral)

Literature Cited

- Allen, C. H., L. Parrott, and C. Kyle. 2016. An individual-based modelling approach to estimate landscape connectivity for bighorn sheep (*Ovis canadensis*). *PeerJ* **4**:e2001.
- Andrews, K. M. and J. W. Gibbons. 2005. How do highways influence snake movement? Behavioral responses to roads and vehicles. *Copeia* **2005**:772-782.
- Barton, K. A., B. L. Phillips, J. M. Morales, and J. M. Travis. 2009. The evolution of an 'intelligent' dispersal strategy: biased, correlated random walks in patchy landscapes. *Oikos* **118**:309-319.
- Beane, J. C., S. P. Graham, T. J. Thorp, and L. T. Pusser. 2014. Natural history of the southern hognose snake (*Heterodon simus*) in North Carolina, USA. *Copeia* **2014**:168-175.
- Bhupathy, S. and V. S. Vijayan. 1989. Status, distribution and general ecology of the Indian Python, *Python molurus molurus* Linn. in Keoladeo National Park, Bharatpur, Rajasthan. *Journal of the Bombay Natural History Society* **86**:381-387.
- Coulon, A., J. Aben, S. Palmer, V. Stevens, T. Callens, D. Strubbe, L. Lens, E. Matthysen, M. Baguette, and J. Travis. 2015. A stochastic movement simulator improves estimates of landscape connectivity. *Ecology* **96**:2203-2213.
- Crone, E. E. and C. B. Schultz. 2008. Old models explain new observations of butterfly movement at patch edges. *Ecology* **89**:2061-2067.
- DeGregorio, B. A., S. Chiavacci, P. J. Weatherhead, J. D. Willson, T. Benson, and J. H. Sperry. 2014. Snake predation on North American bird nests: culprits, patterns and future directions. *Journal of Avian Biology* **45**:001-009.
- Dorcas, M. E. and J. D. Willson. 2009. Innovative methods for studies of snake ecology and conservation. Pages 5-37 in S. J. Mullin and R. A. Seigel, editors. *Snakes: applied ecology and conservation*. Cornell University Press, Ithaca, NY.
- Dorcas, M. E. and J. D. Willson. 2011. Invasive pythons in the United States: ecology of an introduced predator. University of Georgia Press, Athens.
- Dorcas, M. E. and J. D. Willson. 2013. Hidden giants: problems associated with studying secretive invasive pythons. Pages 367-385 in W. Lutterschmidt, editor. *Reptiles in Research: Investigations of Ecology, Physiology, and Behavior from Desert to Sea*. Nova Science Publ. Inc., Hauppauge, N.Y.
- Dorcas, M. E., J. D. Willson, R. N. Reed, R. W. Snow, M. Rochford, M. A. Miller, W. E. Meshaka, Jr., P. T. Andreadis, F. J. Mazzotti, C. M. Romagosa, and K. M. Hart. 2012. Severe mammal declines coincide with python proliferation in Everglades National Park. *Proceedings of the National Academy of Sciences of the United States of America* **109**:2418-2422.
- Dove, C. J., R. W. Snow, M. R. Rochford, and F. J. Mazzotti. 2011. Birds consumed by the invasive Burmese python (*Python molurus bivittatus*) in Everglades National Park, Florida, USA. *The Wilson Journal of Ornithology* **123**:126-131.
- Enge, K. M. and K. N. Wood. 2002. A pedestrian road survey of an upland snake community in Florida. *Southeastern Naturalist* **1**:365-380.
- Falk, B. G., R. W. Snow, and R. N. Reed. 2016. Prospects and Limitations of Citizen Science in Invasive Species Management: A Case Study with Burmese Pythons in Everglades National Park. *Southeastern Naturalist* **15**:89-102.

- Furman, B. L., B. R. Scheffers, and C. A. Paszkowski. 2011. The use of fluorescent powdered pigments as a tracking technique for snakes. *Herpetological Conservation and Biology* **6**:473-478.
- Gibbons, J. W., V. J. Burke, J. E. Lovich, R. D. Semlitsch, T. D. Tuberville, J. R. Bodie, J. L. Greene, P. H. Niewiarowski, H. H. Whiteman, D. E. Scott, J. H. K. Pechmann, C. R. Harrison, S. H. Bennett, J. D. Krenz, M. S. Mills, K. A. Buhlmann, J. R. Lee, R. A. Seigel, A. D. Tucker, T. M. Mills, T. Lamb, M. E. Dorcas, J. D. Congdon, M. H. Smith, D. H. Nelson, M. B. Dietsch, H. G. Hanlin, J. A. Ott, and D. J. Karapatakis. 1997. Perceptions of species abundance, distribution, and diversity: lessons from four decades of sampling on a government-managed reserve. *Environmental Management* **21**:259-268.
- Gibbons, J. W. and M. E. Dorcas. 2005. *Snakes of the Southeast*. University of Georgia Press, Athens.
- Gibbons, J. W., D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. S. Metts, J. L. Greene, T. Mills, Y. Leiden, S. Poppy, and C. T. Winne. 2000. The global decline of reptiles, déjà vu amphibians. *Bioscience* **50**:653-666.
- Hart, K. M., M. S. Cherkiss, B. J. Smith, F. J. Mazzotti, I. Fujisaki, R. W. Snow, and M. E. Dorcas. 2015. Home range, habitat use, and movement patterns of non-native Burmese pythons in Everglades National Park, Florida, USA. *Animal Biotelemetry* **3**:8.
- Heinrichs, J. A., D. J. Bender, and N. H. Schumaker. 2016. Habitat degradation and loss as key drivers of regional population extinction. *Ecological Modelling* **335**:64-73.
- Jellen, B. C. and M. J. Kowalski. 2007. Movement and growth of neonate eastern massasaugas (*Sistrurus catenatus*). *Copeia* **2007**:994-1000.
- Kingsbury, B. A. and N. J. Robinson. 2016. Movement patterns and telemetry. *Reptile Ecology and Conservation: A Handbook of Techniques*:110.
- Mazzotti, F. J., M. S. Cherkiss, K. M. Hart, R. W. Snow, M. R. Rochford, M. E. Dorcas, and R. N. Reed. 2011. Cold-induced mortality of invasive Burmese pythons in south Florida. *Biological Invasions* **13**:143-151.
- Mazzotti, F. J., M. S. Cherkiss, M. Parry, J. Beauchamp, M. Rochford, B. Smith, K. Hart, and L. A. Brandt. 2016. Large reptiles and cold temperatures: Do extreme cold spells set distributional limits for tropical reptiles in Florida? *Ecosphere* **7**.
- McCleery, R. A., A. Sovie, R. N. Reed, M. W. Cunningham, M. E. Hunter, and K. M. Hart. 2015. Marsh rabbit mortalities tie pythons to the precipitous decline of mammals in the Everglades. *Proceedings. Biological sciences / The Royal Society* **282**.
- McClintock, B. T., R. King, L. Thomas, J. Matthiopoulos, B. J. McConnell, and J. M. Morales. 2012. A general discrete-time modeling framework for animal movement using multistate random walks. *Ecological Monographs* **82**:335-349.
- Means, D. B. 2017. *Diamonds in the Rough: Natural History of the Eastern Diamondback Rattlesnake*. Tall Timbers Press.
- Meshaka, W. E., Jr., W. F. Loftus, and T. Steiner. 2000. The herpetofauna of Everglades National Park. *Florida Scientist* **63**:84-103.
- Miller, G. J., L. L. Smith, S. A. Johnson, and R. Franz. 2012. Home range size and habitat selection in the Florida Pine Snake (*Pituophis melanoleucus mugitus*). *Copeia* **2012**:706-713.

- Morales, J. M., D. T. Haydon, J. Frair, K. E. Holsinger, and J. M. Fryxell. 2004. Extracting more out of relocation data: building movement models as mixtures of random walks. *Ecology* **85**:2436-2445.
- Oldham, C. R., J. Fleckenstein III, W. A. Boys, and S. J. Price. 2016. Enhancing Ecological Investigations of Snakes with Passive Integrated Transponder (PIT) Tag Telemetry. *Herpetological Review* **47**:385-388.
- Parker, W. S. and M. V. Plummer. 1987. Population ecology. Pages 253-301 in R. A. Seigel, J. T. Collins, and S. S. Novak, editors. *Snakes: Ecology and Evolutionary Biology*. The Blackburn Press, Caldwell, New Jersey.
- Patrick, D. A. and J. P. Gibbs. 2009. Snake occurrences in grassland associated with road versus forest edges. *Journal of Herpetology* **43**:716-720.
- Pauli, B. P., N. P. McCann, P. A. Zollner, R. Cummings, J. H. Gilbert, and E. J. Gustafson. 2013. SEARCH: spatially explicit animal response to composition of habitat. *Plos One* **8**:e64656.
- Piou, C., U. Berger, H. Hildenbrandt, V. Grimm, K. Diele, and C. D'Lima. 2007. Simulating cryptic movements of a mangrove crab: recovery phenomena after small scale fishery. *Ecological Modelling* **205**:110-122.
- Platt, D. R. 1969. Natural history of the hognose snakes *Heterodon platirhinos* and *Heterodon nasicus*. University of Kansas Publications of the Museum of Natural History **18**:253-420.
- Reed, R. N., K. M. Hart, G. Rodda, F. J. Mazzotti, R. W. Snow, M. S. Cherkiss, R. Rozar, and S. Goetz. 2011. A field test of attractant traps for invasive Burmese pythons (*Python molurus bivittatus*) in southern Florida. *Wildlife Research* **38**:114-121.
- Reed, R. N. and G. H. Rodda. 2009. Giant constrictors: biological and management profiles and an establishment risk assessment for nine large species of pythons, anacondas, and the boa constrictor. U.S. Geological Survey Open-File Report 2009-1202.
- Reed, R. N. and R. W. Snow. 2014. Assessing risks to humans from invasive Burmese pythons in Everglades National Park, Florida, USA. *Wildlife Society Bulletin* **38**:366-369.
- Reichert, B. E., A. R. Sovie, B. J. Udell, K. M. Hart, R. R. Borkhataria, M. Bonneau, R. Reed, and R. McCleery. 2017. Urbanization may limit impacts of an invasive predator on native mammal diversity. *Diversity and Distributions* **23**:355-367.
- Riitters, K. H. and J. D. Wickham. 2003. How far to the nearest road? *Frontiers in Ecology and the Environment* **1**:125-129.
- Robson, L. E. and G. Blouin-Demers. 2013. Eastern hognose snakes (*Heterodon platirhinos*) avoid crossing paved roads, but not unpaved roads. *Copeia* **2013**:507-511.
- Rochford, M., K. L. Krysko, J. Nifong, L. Wilkins, R. W. Snow, and M. S. Cherkiss. 2010. *Python molurus bivittatus* (Burmese Python). Diet. *Herpetological Review* **41**:97.
- Rodda, G. 2012. Population Size and Demographics. Pages 283-322 in R. W. McDiarmid, M. S. Foster, C. Guyer, J. W. Gibbons, and N. Chernoff, editors. *Reptile Biodiversity: Standard Methods for Inventory and Monitoring*. University of California Press, Berkeley.
- Rodda, G. H., C. S. Jarnevich, and R. N. Reed. 2009. What parts of the US mainland are climatically suitable for invasive alien pythons spreading from Everglades National Park? *Biological Invasions* **11**:241-252.
- Rodda, G. H., C. S. Jarnevich, and R. N. Reed. 2011. Challenges in Identifying Sites Climatically Matched to the Native Ranges of Animal Invaders. *Plos One* **6**:1-18.

- Rupp, S. P. and P. Rupp. 2010. Development of an individual-based model to evaluate elk (*Cervus elaphus nelsoni*) movement and distribution patterns following the Cerro Grande Fire in north central New Mexico, USA. *Ecological Modelling* **221**:1605-1619.
- Schwarzkopf, L. and R. A. Alford. 2002. Nomadic movement in tropical toads. *Oikos* **96**:492-506.
- Shepard, D., A. Kuhns, M. Dreslik, and C. Phillips. 2008. Roads as barriers to animal movement in fragmented landscapes. *Animal Conservation* **11**:288-296.
- Siers, S. R., J. A. Savidge, and R. N. Reed. 2014. Invasive Brown Treesnake Movements at Road Edges Indicate Road-Crossing Avoidance. *Journal of Herpetology* **48**:500-505.
- Snow, R. W., M. L. Brien, M. S. Cherkiss, L. Wilkins, and F. J. Mazzotti. 2007a. Dietary habits of the Burmese python, *Python molurus bivittatus*, in Everglades National Park, Florida. *Herpetological Bulletin* **101**:5-7.
- Snow, R. W., K. L. Krysko, K. M. Enge, L. Oberhofer, A. Warren-Bradley, and L. Wilkins. 2007b. Introduced populations of *Boa constrictor* (Boidae) and *Python molurus bivittatus* (Pythonidae) in southern Florida. Pages 416-438 in R. W. Henderson and R. Powell, editors. *Biology of the Boas and Pythons*. Eagle Mountain Publishing, Eagle Mountain, Utah.
- Sovie, A. R., R. A. McCleery, R. J. Fletcher, and K. M. Hart. 2016. Invasive pythons, not anthropogenic stressors, explain the distribution of a keystone species. *Biological Invasions* **18**:3309-3318.
- Steen, D. A. 2010. Snakes in the grass: secretive natural histories defy both conventional and progressive statistics. *Herpetological Conservation and Biology* **5**:183-188.
- Steen, D. A., C. J. McClure, W. B. Sutton, D. C. Rudolph, J. B. Pierce, J. R. Lee, L. L. Smith, B. B. Gregory, D. L. Baxley, and D. J. Stevenson. 2014. Copperheads are common when kingsnakes are not: relationships between the abundances of a predator and one of their prey. *Herpetologica* **70**:69-76.
- Steen, D. A. and L. L. Smith. 2009. Eastern kingsnake (*Lampropeltis getula getula*) home ranges exhibit limited overlap. *Southeastern Naturalist* **8**:553-558.
- Sullivan, B. K. 2000. Long-term shifts in snake populations: a California site revisited. *Biological Conservation* **94**:321-325.
- Todd, B. D., J. D. Willson, and J. W. Gibbons. 2010. The global status of reptiles and causes of their decline. Pages 47-67 in D. Sparling, G. Linder, C. Bishoip, and S. Krest, editors. *Ecotoxicology of Reptiles and Amphibians*. SETAC Press, Pensacola, FL.
- Tozetti, A. M. and M. Martins. 2007. A technique for external radio-transmitter attachment and the use of thread-bobbins for studying snake movements. *South American Journal of Herpetology* **2**:184-190.
- Tuberville, T. D., J. R. Bodie, J. B. Jensen, L. LaClaire, and J. W. Gibbons. 2000. Apparent decline of the southern hog-nosed snake, *Heterodon simus*. *Journal of the Elisa Mitchell Scientific Society* **116**:19-40.
- Turchin, P. 1998. *Quantitative analysis of movement: measuring and modeling population redistribution in animals and plants*. Sinauer Associates Sunderland.
- Wang, M. and V. Grimm. 2007. Home range dynamics and population regulation: an individual-based model of the common shrew *Sorex araneus*. *Ecological Modelling* **205**:397-409.

- Ward, M. P., J. H. Sperry, and P. J. Weatherhead. 2013. Evaluation of automated radio telemetry for quantifying movements and home ranges of snakes. *Journal of Herpetology* **47**:337-345.
- Willson, J. D. 2016. Surface-dwelling reptiles. Pages 125-138 *in* C. K. Dodd, editor. *Reptile Ecology and Conservation: A Handbook of Techniques*. Oxford University Press, Oxford, UK.
- Willson, J. D., M. E. Dorcas, and R. W. Snow. 2011. Identifying plausible scenarios for the establishment of invasive Burmese pythons (*Python molurus*) in southern Florida. *Biological Invasions* **13**:1493-1504.
- Willson, J. D. and C. T. Winne. 2016. Evaluating the functional importance of secretive species: A case study of aquatic snake predators in isolated wetlands. *Journal of Zoology* **298**:266-273.
- Zollner, P. A. and S. L. Lima. 1999. Search strategies for landscape-level interpatch movements. *Ecology* **80**:1019-1030.

Tables

Table 1. Systematic road survey data for *Heterdon simus* for nine years in the North Carolina Sandhills. Data adapted from Beane et al. 2014.

Year	# Dates 1 Sept -15 Nov	# Survey hrs.	Live <i>H. simus</i> Detected	Encounters per h
1996	17	80	14	0.18
1998	36	102	2	0.02
2000	25	83	7	0.08
2002	18	56	7	0.13
2005	28	60	1	0.02
2007	30	95	8	0.08
2009	34	76	9	0.12
2011	21	42	4	0.10
2012	27	62	2	0.03
Total	236	656	54	
			Mean	0.083
			SD	0.053

Table 2. Data for timed natural road crossing events of *Heterodon simus*.

Date	Location	Snake Total Length (cm)	Road Type	Distance Crawled (cm)	Time (sec)	Speed (cm/sec)	Time to Cross 550 cm Road
5/29/2014	SC	48	paved	270	180	1.5	6.64
10/18/2014	SC	49	paved	300	229	1.3	7.62
10/18/2014	SC	20	paved	269	93	2.9	3.28
10/19/2014	SC	19	paved	95	105	0.9	10.48
10/19/2014	SC	43	paved	70	52	1.3	7.34
10/24/2014	NC	18	paved	162	90	1.8	5.26
10/12/2014	FL	47	sand	145	91	1.6	6.24
10/24/2014	FL	19	sand	24	39	0.6	15.41
10/22/2014	FL	18	paved	45	33	1.4	6.94
Mean						1.5	7.69
SD						0.6	3.47

Table 3. Systematic road survey data for *Python molurus* on the Main Park Road in Everglades National Park (ENP) from 2003-2017, amassed from multiple data sources. “Agents” refer to citizen scientists permitted by the ENP ‘Authorized Agent’ Program (Falk et al. 2016).

Source	Year	# Dates 1 June - 30 Nov	# Survey hrs.	# Survey km	Live <i>P. molurus</i> Encountered	Encounters per hr.
Snow	2003	6	22.5	1,082	1	0.04
Snow	2004	14	42.3	2,029	5	0.12
Snow	2005	7	21.6	1,035	3	0.14
Snow	2006	9	23.8	1,141	10	0.42
Willson	2006	2	11.7	562	0	0.00
Smith	2009	8	32.5	1,344	3	0.09
Willson	2009	5	31.7	1,668	5	0.16
Miller	2010	39	171.9	8,733	6	0.03
Smith	2010	7	22.5	1,133	3	0.13
Miller	2011	41	144.7	7,648	10	0.07
Willson	2013	12	45.4	2,574	3	0.07
Willson	2014	4	23.3	1,290	0	0.00
Agents	2014	25	89.9	3,882	8	0.09
Agents	2015	74	361.3	12,267	22	0.06
USGS	2015	181	499.8	23,753	15	0.03
Willson	2015	19	84.2	4,554	8	0.09
Smith	2015	37	156.3	6,462	6	0.04
Willson	2016	7	28.7	1,328	4	0.14
Agents	2016	23	113.2	3,282	4	0.04
Agents	2017	11	38.2	1,429	3	0.08
Willson	2017	11	43.7	2,302	6	0.14
Total		542	2009.0	89,498	125	

Table 4. Data for timed natural road crossing events of *Python molurus* in Everglades National Park, FL. Time detectable on road was calculated as (667 cm + Snake Total Length / Crawling Speed).

Date	Sex	Snake Total Length (cm)	Distance Crawled (cm)	Time (sec)	Crawling	Time detectable on 667 cm Road (min)
					Speed (cm/sec)	
9/10/2010	U	213	330	110	3.00	4.89
5/27/2013	F	201	201	65	3.12	4.64
6/6/2013	M	196	196	29	6.77	2.12
8/13/2014	M	82	82	19	4.32	2.89
8/13/2014	F	74	178	54	3.32	3.72
8/15/2014	F	77	185	90	2.06	6.04
8/17/2014	M	75	75	80	0.94	13.18
8/18/2014	M	84	84	22	3.78	3.31
8/18/2014	M	62	62	17	3.68	3.30
8/18/2014	M	74	74	23	3.26	3.79
8/19/2014	F	167	295	152	1.94	7.16
8/19/2014	M	79	71	34	2.09	5.95
10/14/2014	M	199	199	62	3.21	4.50
11/6/2014	M	115	115	54	2.13	6.12
11/23/2014	M	160	160	120	1.33	10.33
6/6/2015	M	193	225	38	5.92	2.42
6/9/2015	M	283	330	71	4.65	3.40
6/13/2015	M	206	300	44	6.82	2.13
8/25/2015	M	81	197	36	5.47	2.28
11/14/2015	M	220	225	94	2.39	6.17
11/14/2015	M	197	200	130	1.54	9.36
8/14/2016	M	69	264	179	1.47	8.31
8/15/2016	M	72	309	149	2.07	5.94
8/16/2016	F	68	460	146	3.15	3.89
8/16/2016	F	78	360	101	3.56	3.48
8/16/2016	F	71	305	199	1.53	8.02
8/18/2016	F	221	222	62	3.58	4.13
7/20/2017	F	152	212	70	3.03	4.51
7/24/2017	M	182	320	41	7.86	1.80
7/26/2017	F	178	89	27	3.30	4.27
8/1/2017	F	66	300	282	1.06	11.49
				Mean	3.30	5.28
				SD	1.76	2.87

Table 5. Summary data for the number of predicted python road crossings, based on 1000 random movement paths for each individual, and the number of observed road crossings based on telemetry data. Asterisks indicate pythons which displayed evidence of road attraction.

Python Number	Median	Range	Observed Crossings	Prob < = Observed
1	1	0 - 8	3	0.927
2	1	0-7	0	0.082
3	2	0-9	1	0.464
4	2	0-12	11	0.999*
5	3	0-18	12	0.983*
6	2	0-16	0	0.398
7	0	0-14	0	0.495
8	3	0-16	0	0.083
9	0	0-15	5	0.766
10	3	0-15	0	0.118
11	2	0-10	7	0.967
12	0	0-4	0	0.991
13	0	0-10	1	0.669

Table 6. Suitability of snake species found on DoD lands for road-based density estimation. Table includes all species documented to occur on DoD lands in the continental United States (based on the DoD PARC Herpetofauna Database), attributes that affect their suitability for road-based density estimation (see V. Recommendations for Implementation on DoD Lands), and suitability ranking for each species (1-4 score, with 4 being most suitable). Species attributes based on literature review (Ernst and Ernst 2003) and author's expert opinion. Species most suitable for road-based density estimation are highlighted.

Latin Name	Common Name	Habitat [†]	Telemetry [†]	Road Survey [†]	Active [†]	Ranking [†]
<i>Agkistrodon contortrix</i>	Copperhead	T	Y	Y	N	4
<i>Agkistrodon piscivorus</i>	Cottonmouth	A/T	Y	Y	N	4
<i>Arizona elegans</i>	Glossy Snake	T/F	Y	Y	?	4
<i>Boa constrictor</i>	Boa Constrictor	T/Ar	Y	?	N	4
<i>Bogertophis subocularis</i>	Trans-Pecos Ratsnake	F	Y	Y	N	4
<i>Carphophis amoenus</i>	Common Wormsnake	F	N	N	N	1
<i>Carphophis vermis</i>	Western Wormsnake	F	N	N	N	1
<i>Cemophora coccinea</i>	Scarletsnake	F	N	Y	?	2
<i>Charina bottae</i>	Northern Rubber Boa	T/F	Y	?	N	4
<i>Chilomeniscus stramineus</i>	Variable Sandsnake	F	N	Y	N	2
<i>Chionactis occipitalis</i>	Western Shovel-nosed Snake	F	N	Y	N	2
<i>Clonophis kirtlandii</i>	Kirtland's Snake	A/T	?	N	?	0
<i>Coluber bilineatus</i>	Sonoran Whipsnake	T	Y	N	Y	0
<i>Coluber constrictor</i>	North American Racer	T	Y	Y	Y	3
<i>Coluber flagellum</i>	Coachwhip	T	Y	Y	Y	3
<i>Coluber lateralis</i>	Striped Racer	T	Y	Y	Y	3
<i>Coluber schotti</i>	Schott's Whipsnake	T	Y	Y	Y	3
<i>Coluber taeniatus</i>	Striped Whipsnake	T	Y	Y	Y	3
<i>Contia tenuis</i>	Common Sharp-tailed Snake	F	N	N	N	1
<i>Crotalus adamanteus</i>	Eastern Diamond-backed Rattlesnake	T	Y	Y	N	4
<i>Crotalus atrox</i>	Western Diamond-backed Rattlesnake	T	Y	Y	N	4
<i>Crotalus cerastes</i>	Sidewinder	T	Y	Y	N	4
<i>Crotalus cerberus</i>	Arizona Black Rattlesnake	T	Y	Y	N	4
<i>Crotalus horridus</i>	Timber Rattlesnake	T	Y	Y	N	4
<i>Crotalus lepidus</i>	Rock Rattlesnake	T/F	Y	Y	N	4
<i>Crotalus mitchellii</i>	Speckled Rattlesnake	T	Y	Y	N	4
<i>Crotalus molossus</i>	Black-tailed Rattlesnake	T	Y	Y	N	4

<i>Crotalus oreganus</i>	Western Rattlesnake	T	Y	Y	N	4
<i>Crotalus ornatus</i>	Eastern Black-tailed Rattlesnake	T	Y	Y	N	4
<i>Crotalus pricei</i>	Twin-spotted Rattlesnake	T/F	?	N	N	1
<i>Crotalus ruber</i>	Red Diamond Rattlesnake	T	Y	Y	N	4
<i>Crotalus scutulatus</i>	Mohave Rattlesnake	T	Y	Y	N	4
<i>Crotalus stephensi</i>	Panamint Rattlesnake	T	Y	Y	N	4
<i>Crotalus tigris</i>	Tiger Rattlesnake	T	Y	Y	N	4
<i>Crotalus viridis</i>	Prairie Rattlesnake	T	Y	Y	N	4
<i>Crotalus willardi</i>	Ridge-nosed Rattlesnake	T	?	N	N	1
<i>Diadophis punctatus</i>	Ring-necked Snake	T/F	N	?	N	2
<i>Drymarchon couperi</i>	Eastern Indigo Snake	T	Y	?	Y	3
<i>Drymarchon melanurus</i>	Central American Indigo Snake	T	Y	?	Y	3
<i>Farancia abacura</i>	Red-bellied Mudsake	A	Y	Y	?	3
<i>Farancia erythrogramma</i>	Rainbow Snake	A	Y	?	?	0
<i>Gyalopion canum</i>	Chihuahuan Hook-nosed Snake	F	N	Y	N	2
<i>Haldea striatula</i>	Rough Earthsnake	F	N	N	N	1
<i>Heterodon nasicus</i>	Plains Hog-nosed Snake	T/F	Y	Y	N	4
<i>Heterodon platirhinos</i>	Eastern Hog-nosed Snake	T/F	Y	Y	N	4
<i>Heterodon simus</i>	Southern Hog-nosed Snake	T/F	Y	Y	N	4
<i>Hypsiglena chlorophaea</i>	Desert Nightsnake	T/F	N	Y	N	2
<i>Hypsiglena jani</i>	Chihuahuan Nightsnake	T/F	N	Y	N	2
<i>Hypsiglena ochrorhyncha</i>	Coast Nightsnake	T/F	N	Y	N	2
<i>Lampropeltis californiae</i>	California Kingsnake	T/F	Y	Y	N	4
<i>Lampropeltis calligaster</i>	Yellow-bellied Kingsnake	T/F	Y	Y	N	4
<i>Lampropeltis elapsoides</i>	Scarlet Kingsnake	T/F	N	N	N	1
<i>Lampropeltis extenuata</i>	Short-tailed Kingsnake	F	N	Y	N	2
<i>Lampropeltis gentilis</i>	Western Milksnake	T/F	?	?	N	2
<i>Lampropeltis getula</i>	Eastern Kingsnake	T	Y	?	N	4
<i>Lampropeltis holbrooki</i>	Speckled Kingsnake	T	Y	?	N	4
<i>Lampropeltis nigra</i>	Eastern Black Kingsnake	T	Y	?	N	4
<i>Lampropeltis splendida</i>	Desert Kingsnake	T/F	Y	Y	N	4
<i>Lampropeltis triangulum</i>	Milksnake	T/F	Y	?	N	4
<i>Lampropeltis zonata</i>	California Mountain Kingsnake	T/F	Y	N	N	3
<i>Lichanura orcutti</i>	Northern Three-lined Boa	F	Y	Y	N	4
<i>Lichanura trivirgata</i>	Rosy Boa	F	Y	Y	N	4
<i>Liodytes alleni</i>	Black Swampsnake	A	N	N	N	0
<i>Liodytes rigida</i>	Glossy Swampsnake	A	?	N	N	0
<i>Micruroides euryxanthus</i>	Sonoran Coralsnake	F	N	Y	?	2
<i>Micrurus fulvius</i>	Harlequin Coralsnake	T/F	?	Y	?	2
<i>Micrurus tener</i>	Texas Coralsnake	T/F	?	Y	?	2

<i>Nerodia clarkii</i>	Saltmarsh Watersnake	A	Y	?	?	0
<i>Nerodia cyclopion</i>	Mississippi Green Watersnake	A	Y	?	?	0
<i>Nerodia erythrogaster</i>	Plain-bellied Watersnake	A/T	Y	Y	?	3
<i>Nerodia fasciata</i>	Southern Watersnake	A	Y	Y	?	3
<i>Nerodia floridana</i>	Florida Green Watersnake	A	Y	?	?	0
<i>Nerodia rhombifer</i>	Diamond-backed Watersnake	A	Y	?	?	0
<i>Nerodia sipedon</i>	Common Watersnake	A	Y	?	?	0
<i>Nerodia taxispilota</i>	Brown Watersnake	A	Y	?	?	0
<i>Opheodrys aestivus</i>	Rough Greensnake	T/Ar	N	Y	?	1
<i>Opheodrys vernalis</i>	Smooth Greensnake	T	N	?	?	1
<i>Pantherophis alleghaniensis</i>	Eastern Ratsnake	T/Ar	Y	Y	N	4
<i>Pantherophis emoryi</i>	Great Plains Ratsnake	T/F	Y	Y	N	4
<i>Pantherophis guttatus</i>	Red Cornsnake	T	Y	Y	N	4
<i>Pantherophis obsoletus</i>	Western Ratsnake	T/Ar	Y	Y	N	4
<i>Pantherophis ramspotti</i>	Western Foxsnake	T	Y	Y	N	4
<i>Pantherophis slowinskii</i>	Slowinski's Cornsnake	T	Y	Y	N	4
<i>Pantherophis spiloides</i>	Gray Ratsnake	T/Ar	Y	Y	N	4
<i>Pantherophis vulpinus</i>	Eastern Foxsnake	T	Y	Y	N	4
<i>Phyllorhynchus browni</i>	Saddled Leaf-nosed Snake	F	?	Y	N	2
<i>Phyllorhynchus decurtatus</i>	Spotted Leaf-nosed Snake	F	N	Y	N	2
<i>Pituophis catenifer</i>	Gophersnake	T/F	Y	Y	N	4
<i>Pituophis melanoleucus</i>	Pinesnake	T/F	Y	Y	N	4
<i>Pituophis ruthveni</i>	Louisiana Pinesnake	T/F	Y	Y	N	4
<i>Python molurus bivittatus</i>	Burmease Python	T	Y	Y	N	4
<i>Ramphotyphlops braminus</i>	Brahminy Blindsnake	F	N	N	N	0
<i>Regina grahamii</i>	Graham's Crayfish Snake	A	?	?	N	1
<i>Regina septemvittata</i>	Queensnake	A	?	N	N	0
<i>Rena dissecus</i>	New Mexico Threadsnake	F	N	?	N	0
<i>Rena dulcis</i>	Texas Threadsnake	F	N	?	N	0
<i>Rena humilis</i>	Western Threadsnake	F	N	?	N	0
<i>Rhadinaea flavilata</i>	Pine Woods Littersnake	F	N	N	N	1
<i>Rhinocheilus lecontei</i>	Long-nosed Snake	F	Y	Y	?	4
<i>Salvadora grahamiae</i>	Eastern Patch-nosed Snake	T	Y	Y	Y	3
<i>Salvadora hexalepis</i>	Western Patch-nosed Snake	T	Y	Y	Y	3
<i>Sistrurus catenatus</i>	Massasauga	T	Y	?	N	4
<i>Sistrurus miliarius</i>	Pygmy Rattlesnake	T	?	Y	N	2
<i>Sonora semiannulata</i>	Western Groundsnake	F	N	?	N	2
<i>Storeria dekayi</i>	Dekay's Brownsnake	T	N	Y	N	2
<i>Storeria</i>	Red-bellied Snake	T/F	N	N	N	1

<i>occipitomaculata</i>						
<i>Storeria victa</i>	Florida Brownsnake	T	N	Y	N	2
<i>Tantilla coronata</i>	Southeastern Crowned Snake	F	N	N	N	1
<i>Tantilla gracilis</i>	Flat-headed Snake	F	N	N	N	1
<i>Tantilla hobartsmithi</i>	Smith's Black-headed Snake	F	N	Y	N	2
<i>Tantilla nigriceps</i>	Plains Black-headed Snake	F	N	Y	N	2
<i>Tantilla planiceps</i>	Western Black-headed Snake	F	N	Y	N	2
<i>Tantilla relicta</i>	Florida Crowned Snake	F	N	N	N	1
<i>Thamnophis atratus</i>	Aquatic Gartersnake	A/T	?	?	?	1
<i>Thamnophis butleri</i>	Butler's Gartersnake	T	?	?	?	1
<i>Thamnophis cyrtopsis</i>	Black-necked Gartersnake	A/T	?	?	?	1
<i>Thamnophis elegans</i>	Terrestrial Gartersnake	T	Y	Y	?	4
<i>Thamnophis eques megalops</i>	Mexican Gartersnake	A/T	?	?	?	1
<i>Thamnophis hammondi</i>	Two-striped Gartersnake	A/T	?	?	?	1
<i>Thamnophis marcianus</i>	Checkered Gartersnake	T	Y	Y	?	4
<i>Thamnophis ordinoides</i>	Northwestern Gartersnake	T	?	?	?	1
<i>Thamnophis proximus</i>	Western Ribbonsnake	T	?	?	?	1
<i>Thamnophis radix</i>	Plains Gartersnake	T	?	?	?	1
<i>Thamnophis sauritus</i>	Eastern Ribbonsnake	T	?	?	?	1
<i>Thamnophis sirtalis</i>	Common Gartersnake	T	Y	Y	?	4
<i>Trimorphodon lambda</i>	Sonoran Lyresnake	T/F	?	Y	N	2
<i>Trimorphodon lyrophanes</i>	California Lyresnake	T/F	?	Y	N	2
<i>Trimorphodon wilkinsonii</i>	Texas Lyresnake	T/F	?	Y	N	2
<i>Tropidoclonion lineatum</i>	Lined Snake	T/F	N	?	?	1
<i>Virginia valeriae</i>	Smooth Earthsnake	F	N	N	N	1

[†]**Definitions:**

Habitat: T = terrestrial; F = Fossorial; A = aquatic; Ar = Arboreal. Terrestrial and Fossorial species are most ideal for road-based density estimation.

Telemetry (Y/N/?): Is the species large enough for current implantable radio-transmitters. ? = only some individuals suitable for transmitters with short battery life.

Road Survey (Y/N/?): Are road surveys a viable method for sampling the species. Y = frequently captured using road surveys at most locations where species occurs; ? = captured frequently during road surveys at some locations; N = generally not captured using road surveys

Active (Y/N/?): Does the species make frequent or extensive movements on a daily basis. ? = spatial movement patterns are not well known.

Ranking (0-4): 0 = biology not suitable; 1 = biology possible, not suitable for telemetry; 2 = biology ideal, not suitable for telemetry; 3 = biology possible, suitable for telemetry; 4 = biology ideal, suitable for telemetry.

Figures



Fig. 1. Southern Hognose Snake (*Heterodon simus*), a rare upland snake that is most often encountered while crossing roads. Photos by J.D. Willson.

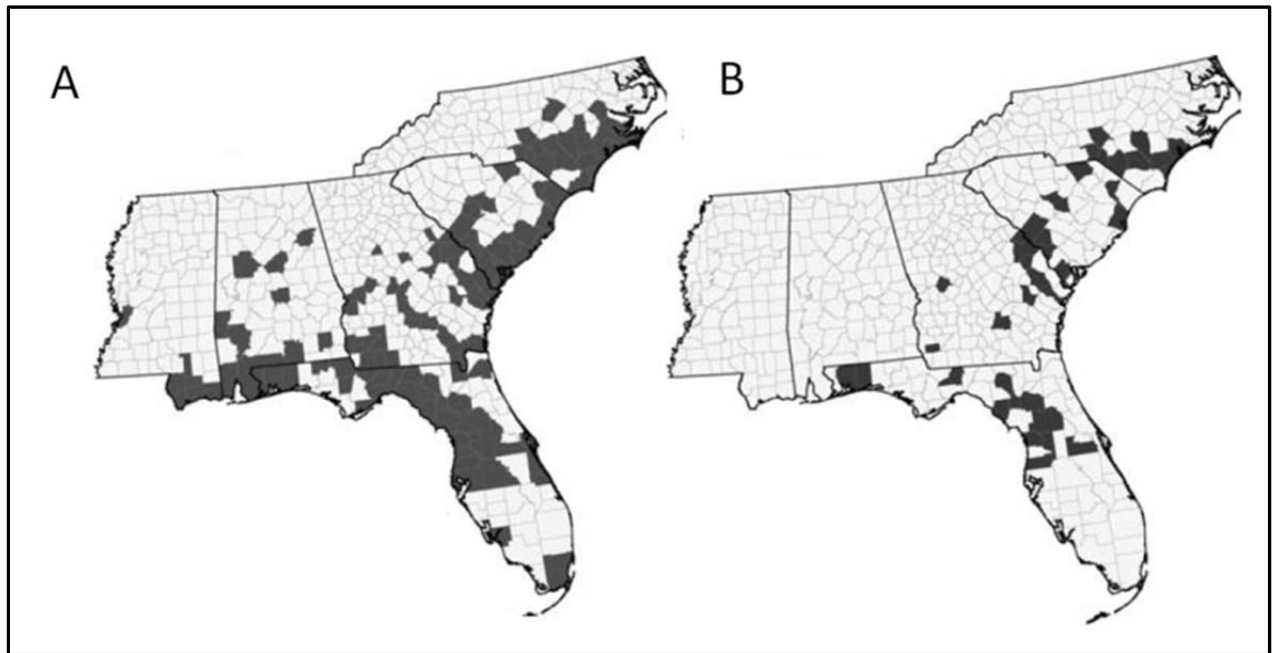


Fig. 2. Past and current distribution of *Heterodon simus*. A) Distribution of all documented occurrences (by county); B) Distribution of all known occurrences since 1985. Figure adapted from Tuberville et al. 2000.

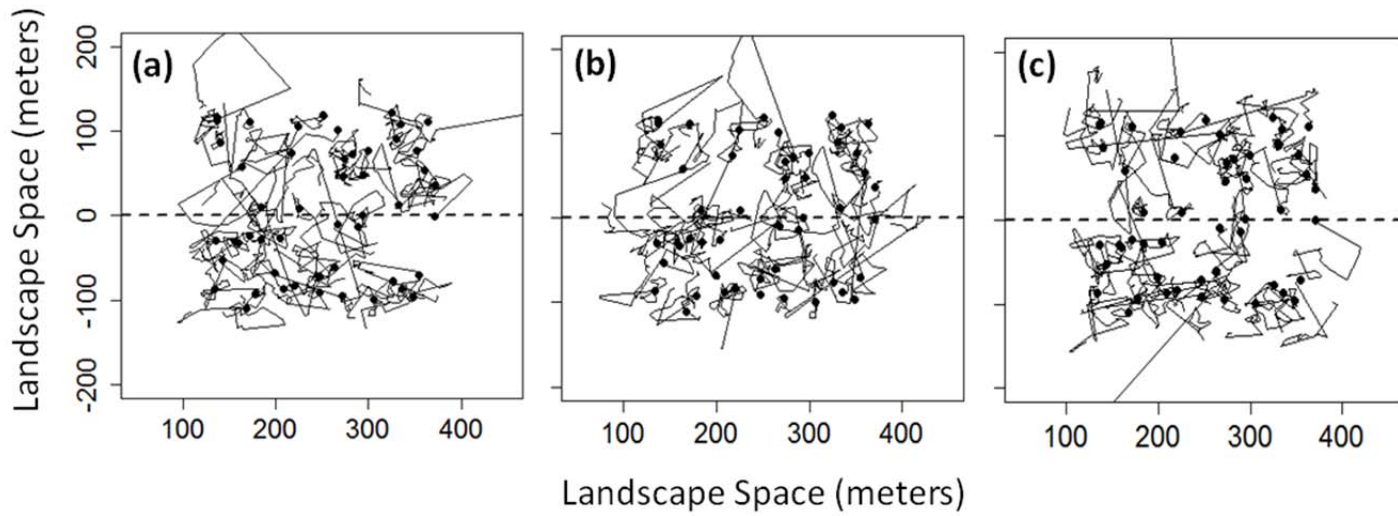


Fig. 3. Examples of movement paths of simulated snakes generated using individual-based spatial movement model parameterized using a) no road bias (0, number of road crossings = 28), b) strong road attraction (0.3, number of road crossings = 46), and c) strong road avoidance (0.3, number of road crossings = 11). Paths crossing the horizontal dashed line represent road crossing events.

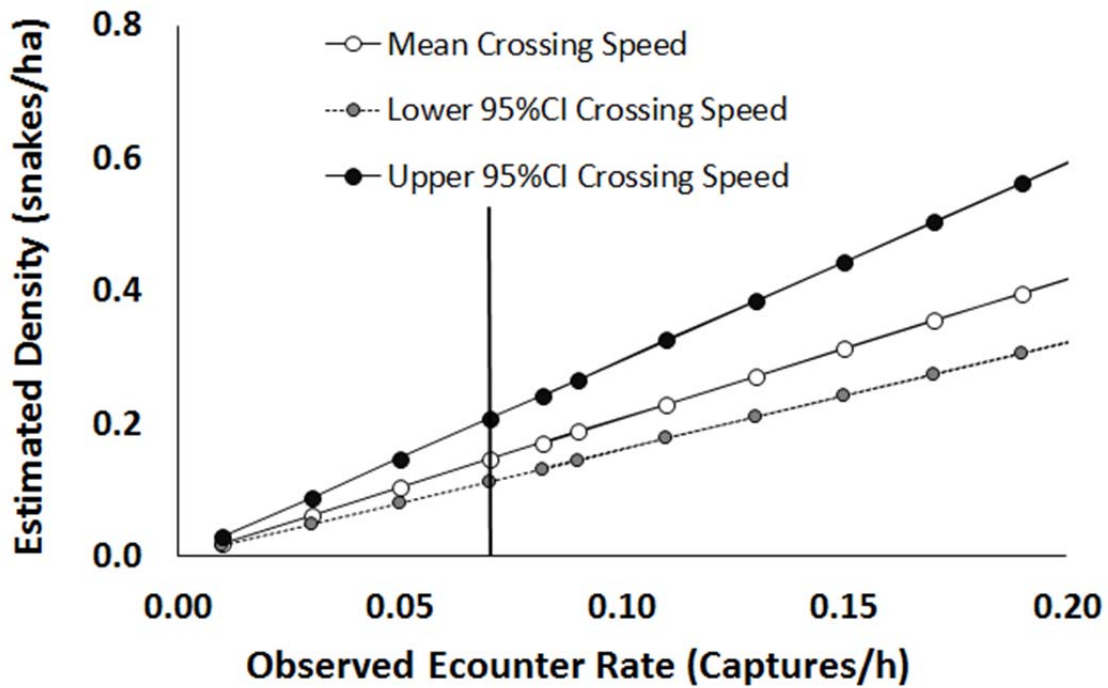


Fig. 4. Relationship between encounter rate and estimated density of *Heterodon simus*, bounded by the 95% confidence interval of road crossing speeds observed in behavioral observations. Vertical bold line indicates mean observed encounter frequency (0.082 live snakes per h) across 656 hours of survey time over nine years in the North Carolina Sandhills. Thus, mean estimated density is 0.17 snakes per ha.

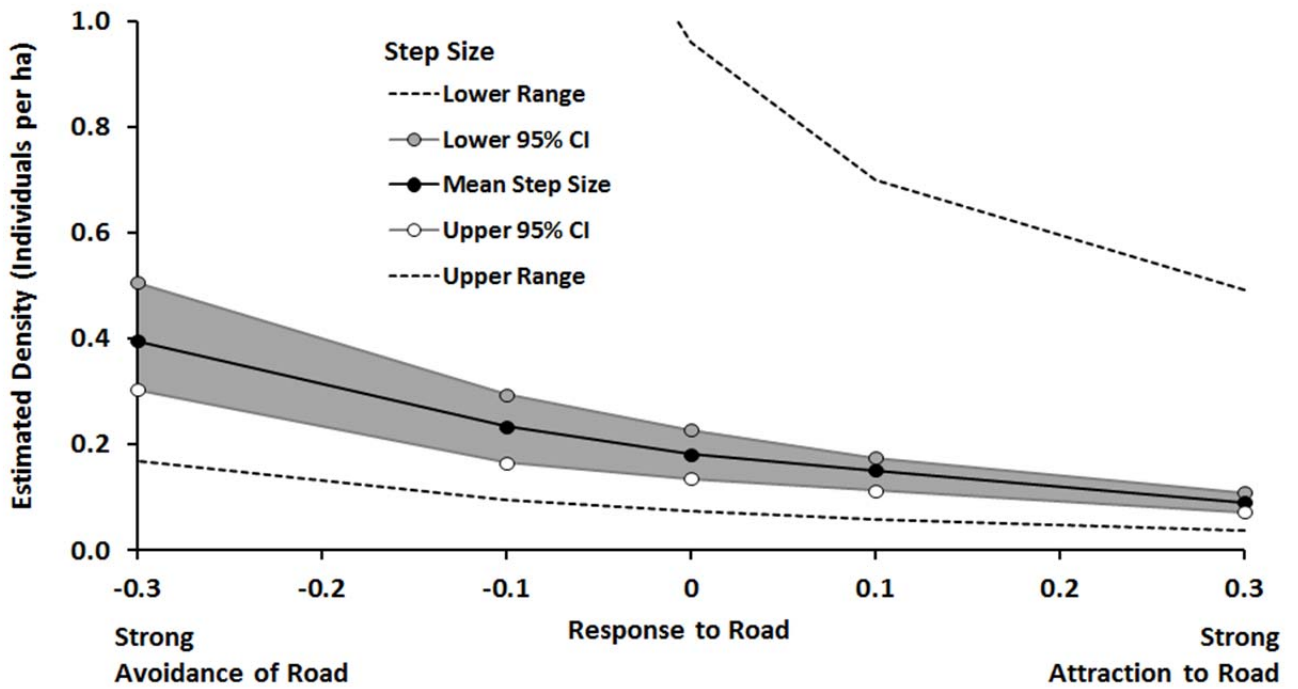


Fig. 5. Sensitivity of estimated snake density to assumptions about step size and road bias.

Stronger road avoidance yielded greater density estimates than road attraction. Step size also impacted estimated density, with smaller step sizes yielding greater density estimates.



Fig. 6. Burmese Python (*Python molurus bivittatus*), an exotic species that has invaded southern Florida and appears to be severely affecting wildlife populations in the region. Photos by J.D. Willson.

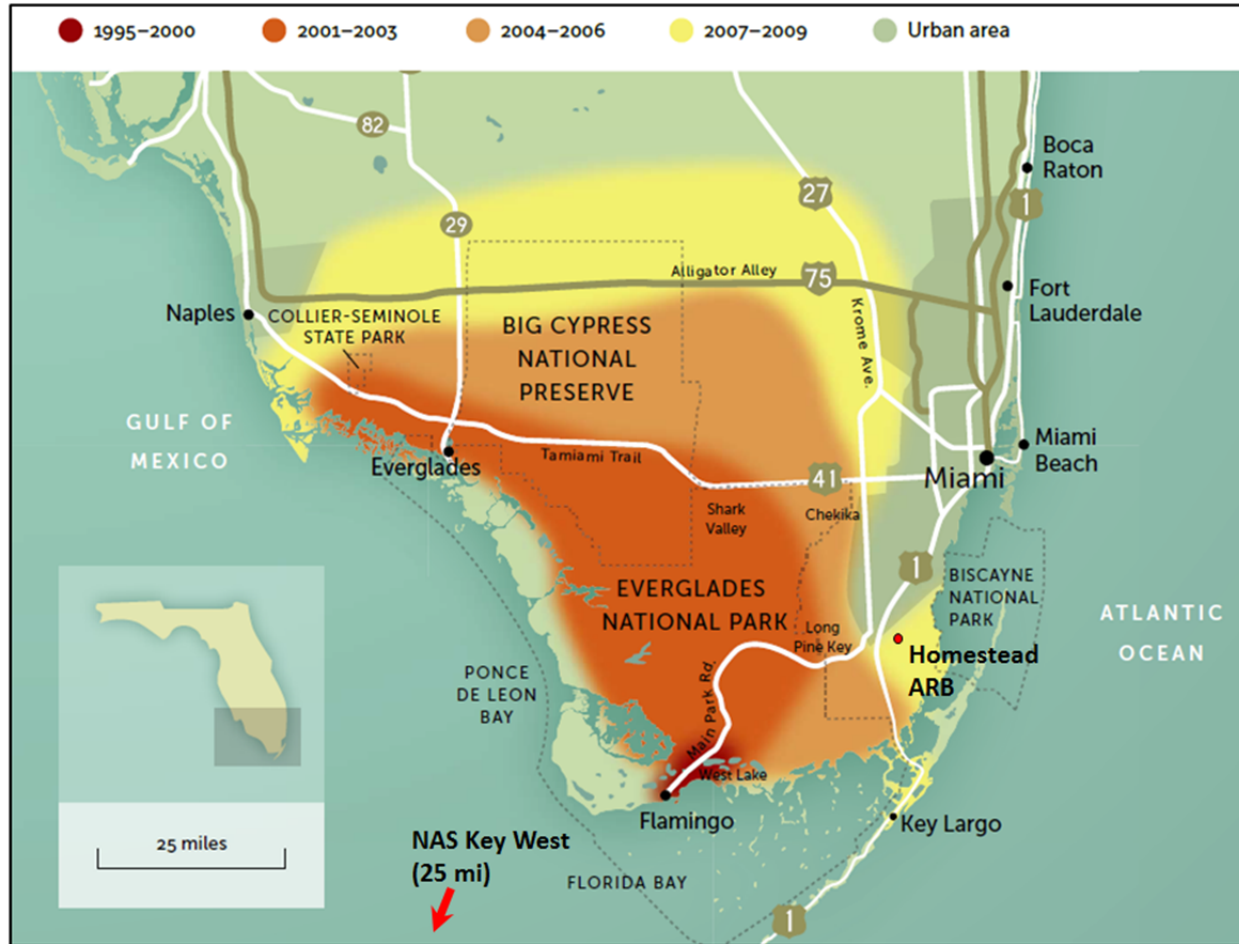


Fig. 7. Spatial distribution of *Python molurus* in Florida between 1995 and 2009. Python density estimation for this project focused on the Main Park Road, between Long Pine Key and Flamingo, which is the core of the python's invasive range. Locations of DoD Lands (Homestead ARB and NAS Key West) in the region are indicated. Figure adapted from Dorcas and Willson (2011).

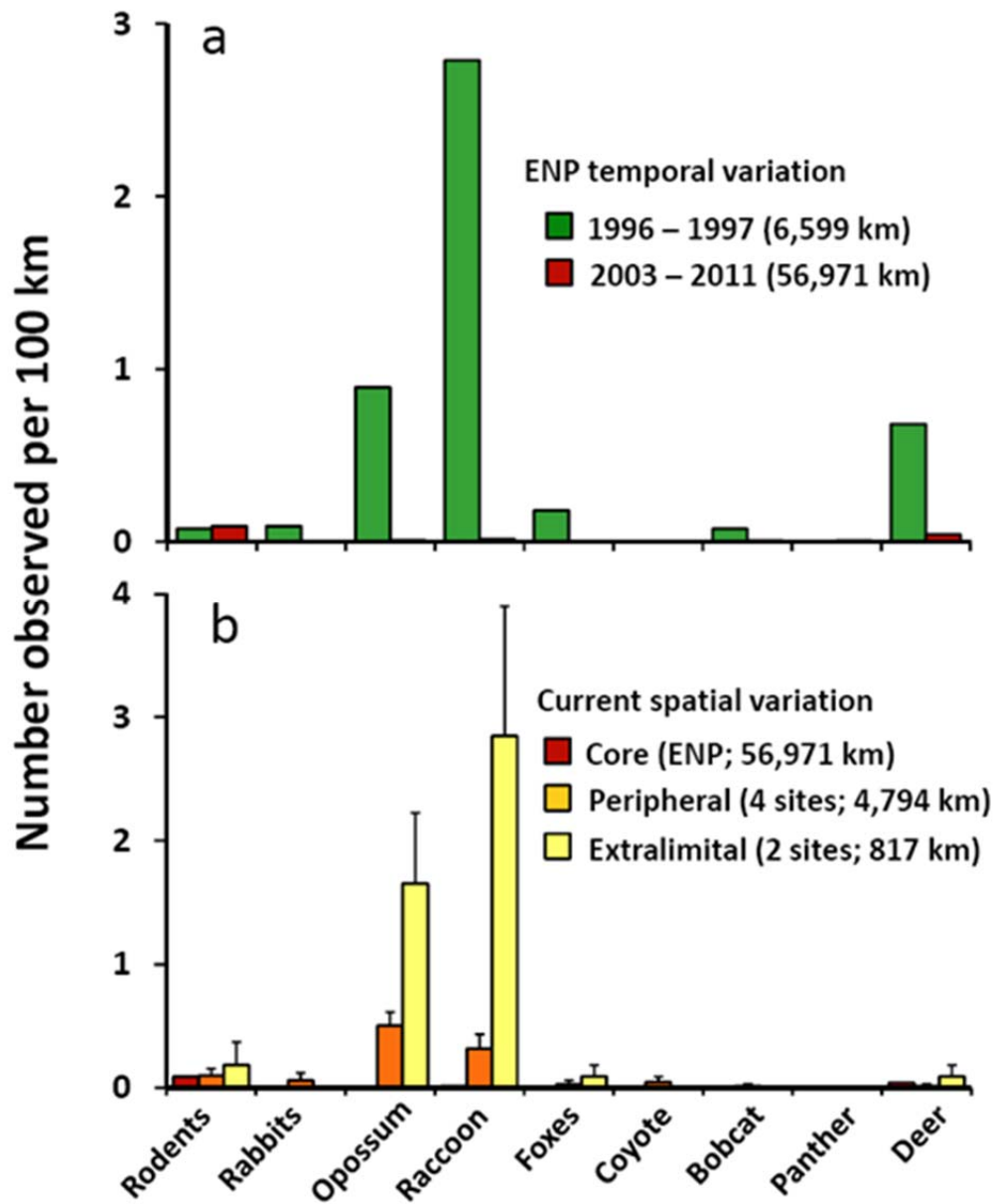


Fig. 8. Variation in mammal abundances in South Florida in relation to python invasion. (a) Temporal variation in mammal encounter rates in Everglades National Park (ENP), as reflected in distance-corrected road survey counts before (1996-1997) and after (2003-2011) pythons become common. (b) Current spatial variation in mammal encounter rates in core (ENP), peripheral (n=4 sites), and extralimital (n=2 sites) regions of python range. Pythons have been recorded in the core region for at least a decade and in peripheral locations more recently. Figure adapted from Dorcas et al. (2012).

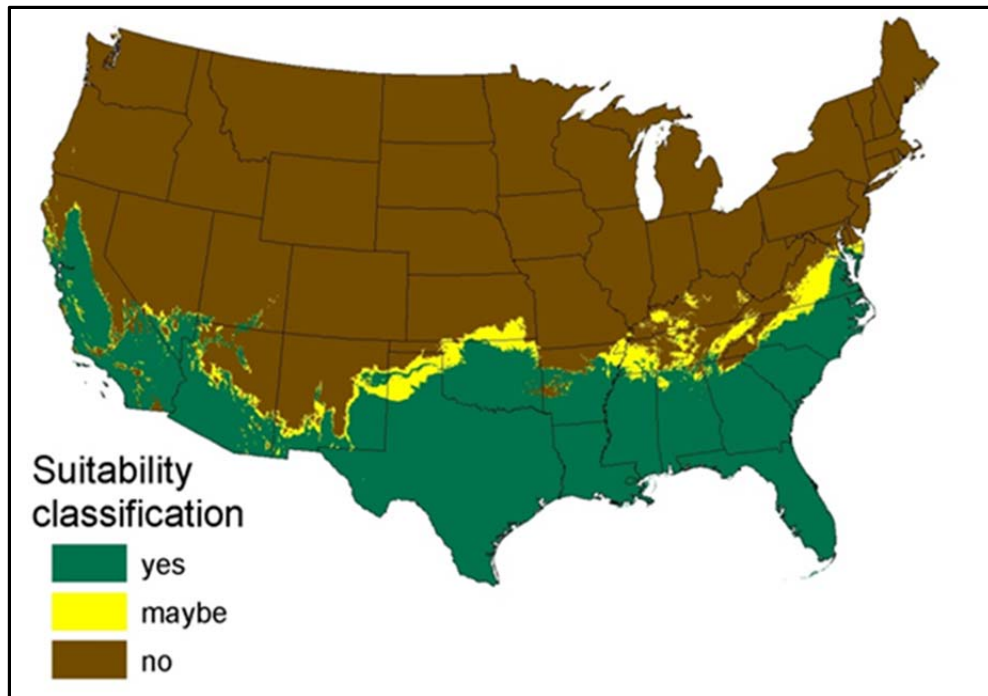


Fig. 9. Areas of the United States predicted to have suitable climate for *Python molurus*, based on climate matching models that considered temperature and precipitation. Figure adapted from Rodda et al. (2009).

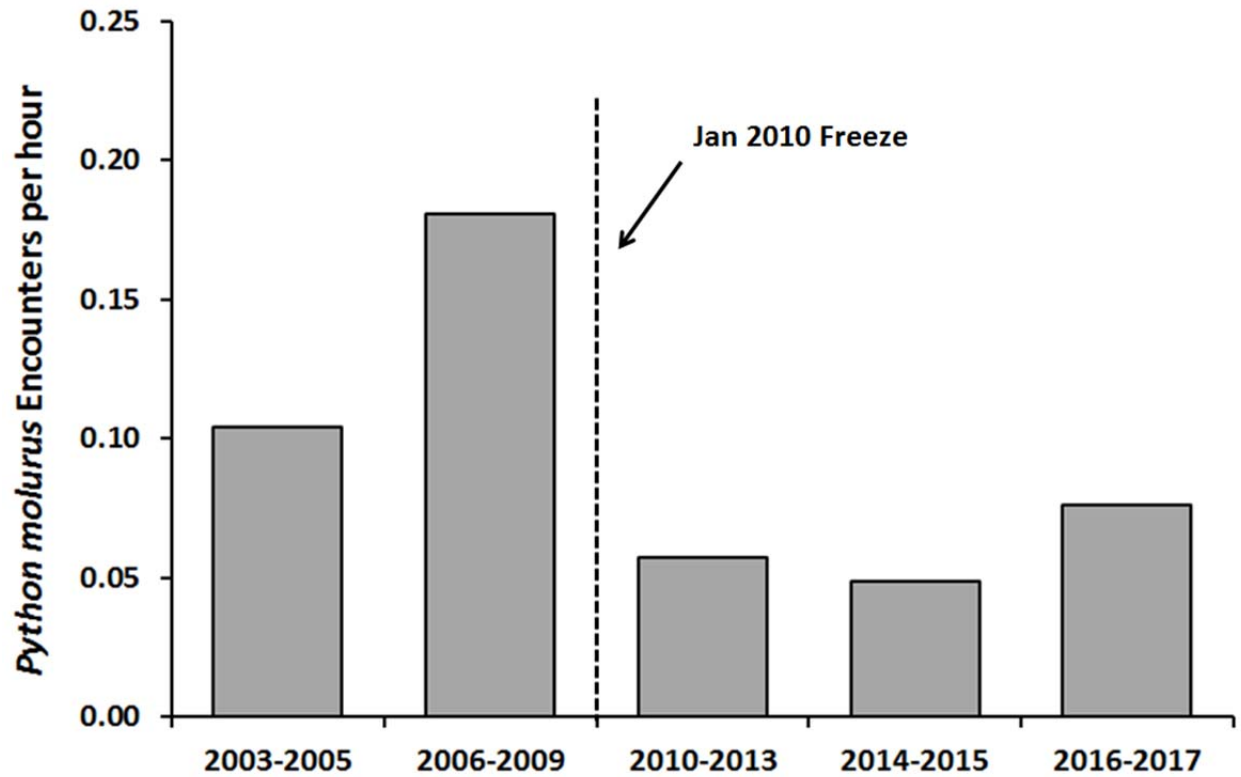


Fig. 10. Effort-corrected encounter rates of *Python molurus* during systematic road surveys (N = 542 surveys, totaling 2009 h, 89,498 km) of the Main Park Road in Everglades National Park from 2003 – 2017. Surveys were conducted between 1 June and 30 November. Years are grouped by availability of data (Table 4) and important events, particularly relative to a severe freeze that occurred in ENP in Jan 2010 (dotted line).

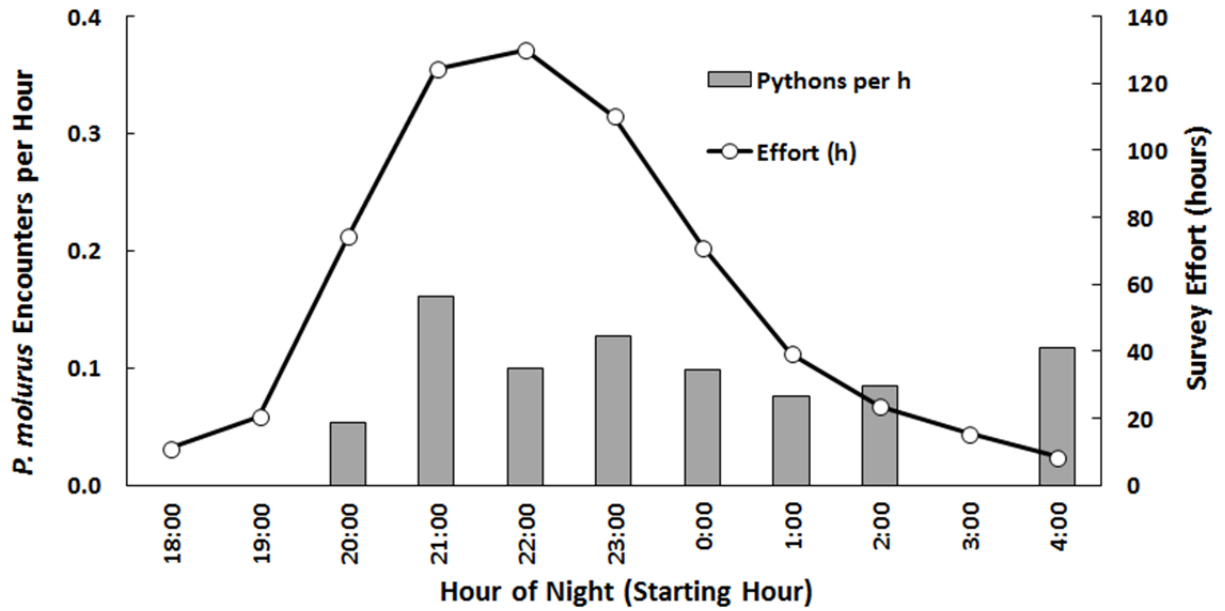


Fig. 11. Diel patterns of python road crossing activity (effort-corrected encounter rate) relative to survey effort for surveys where time of python captures was recorded. Note that although survey activity was concentrated between 20:00 and 01:00 h, python capture rates were relatively uniform throughout the night.

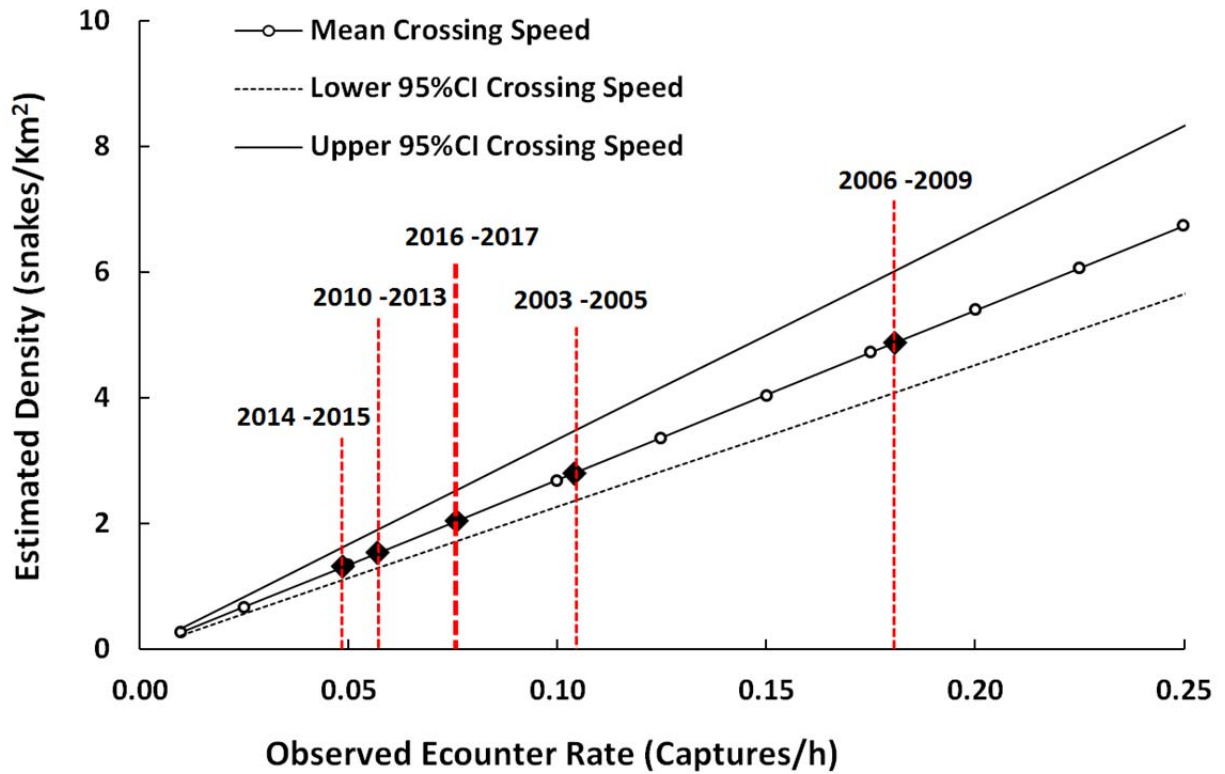


Fig. 12. Relationship between encounter rate and estimated density of *P. molurus* in Everglades National Park (ENP), bounded by the 95% confidence interval of road crossing speeds observed in behavioral observations. Vertical dashed lines indicate mean observed encounter frequencies during year intervals on the Main Park Road in ENP, based on 2009 h of survey effort across 542 nights. Bold dashed vertical line represents current (2016-2107) encounter rate, which corresponds to a mean estimated density of 2.05 pythons per km², assuming a neutral road response.

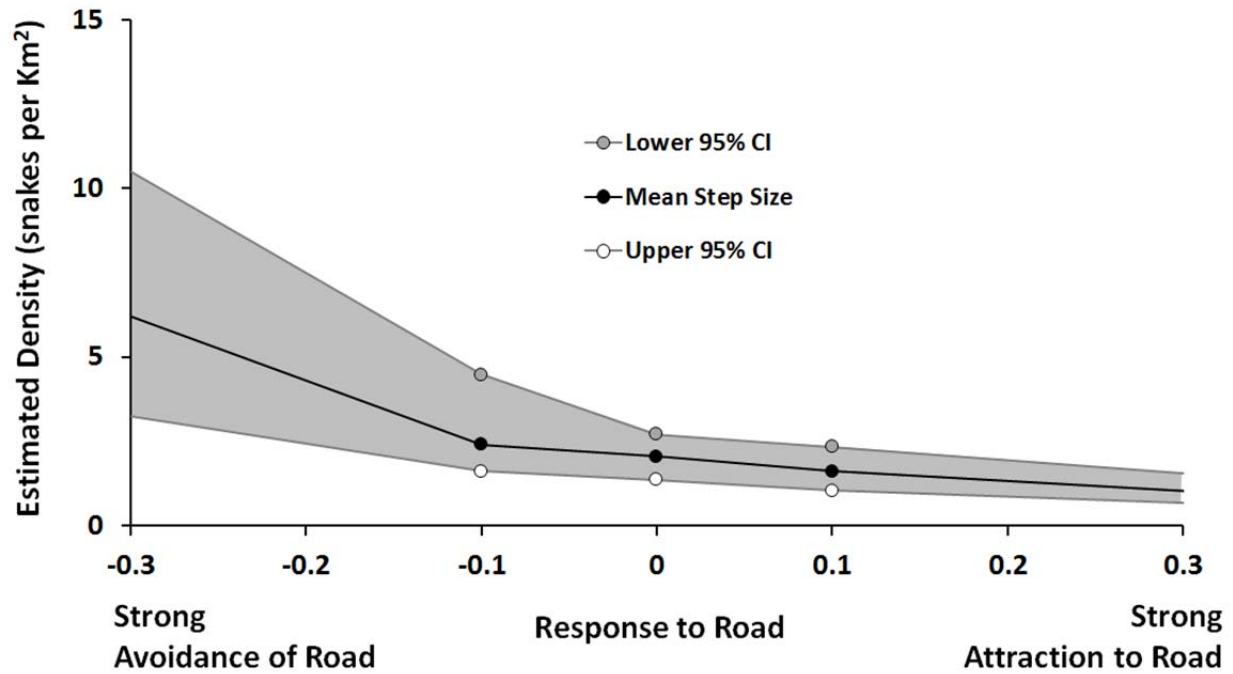


Fig. 13. Sensitivity of current (2016-2017) estimated *P. molurus* density to model assumptions about step size and road bias. Stronger road avoidance yielded greater density estimates than road attraction. Step size also affected estimated density, with smaller step sizes yielding greater density estimates.